

From too little money to too much?

The annual allocation of funds between the British research councils is a more cheerful event this year than in the recent past. But money by itself will not put British research back on its feet.

If it was true in the early 1980s that “£500 million is a lot of money”, then how much more is £897 million now? The numbers are the sums available to the British research councils in 1980 and in the financial year beginning next April (see page 199). The second sum will be further augmented by the transfer of £70 million from the recurrent budget of the universities. The quotation is that with which the Prime Minister, Mrs Margaret Thatcher, dealt with those who used to complain that British science was starved of funds. Apocryphal or otherwise, Thatcher’s riposte was always correct, while now the best part of £1,000 million is indeed a lot of money. But that only goes to show that what is wrong with British science is not that funds are hard to come by.

Ten years ago, the outstanding trouble was that a previous decade of restraint had left too little slack in the support system to allow bold initiatives a decent chance of attracting funds. The past decade has created two further causes of decline — the diversion of funds that might have been spent on research to the premature retirement of able people and the continuing distraction of academic researchers by the impoverishment and enforced upheaval of their institutions. That is why the prospect that there will now be a real (as distinct from inflationary) increase in the funds available for research in Britain, welcome though it is, will not put the British research enterprise to rights. Uncertainty persists in many important ways, too great a proportion of the funds now available is pre-assigned and the unambitious tradition of the past decade exerts a baleful influence.

Uncertainty? On the face of things, now that major questions such as continued British membership of the European high-energy physics laboratory at Geneva (called CERN) have been settled, what doubts can there be in people’s minds about the future? Sadly, there are several, of which the most conspicuous lies most squarely at the British government’s own door. It is now nearly a year since a committee appointed by the Advisory Board for the Research Councils (ABRC) recommended that the five existing research councils should be merged. The objective was to settle boundary disputes between the councils, notably the wish of the Medical Research Council to be predominantly responsible for supporting research in biology, application as well as discovery. Much of 1989 was given over to argument over this proposal. ABRC’s opinion was delivered to the government

the same year. But nothing has yet been decided. Whitehall complains that science cannot take decisions for itself, but the government is no better.

The over-commitment of resources to fixed enterprises is little changed. Of the £76 million extra available next year, only £1.7 million will be spent on research grants in what is called the research councils’ “responsive mode” (which means that they have not themselves specified the topics). And three-quarters of the extra £11 million to be spent next year on manpower training will be used to increase the stipends of government-supported research students by a long overdue £600 a year. There is no vision, in ABRC’s advice, of a time when it will again be possible for people, most probably young people, to recruit help, and the funds with which to pay for it, in the pursuit of a daring project in research.

That, of course, is the tradition of the past decade with which British science is now lumbered. It is understandable, after a decade in which mere survival seemed at risk, that the preservation of each and every going concern should seem worthwhile. The fault, in such a policy, is that good ideas (still plentiful) and excellent research (which still abounds) fail to make a mark in an increasingly competitive environment, now more than ever. The managers of the research enterprise have been slow to recognize this truth. They should appreciate that one of their most urgent needs is to find a few fields of enquiry in which British science remains expert and in which, with generous support, it could win the reputation of being preeminent. But why? Not for kudos, or out of altruism, but from the simple recognition that the flight of young people from science on leaving British schools and universities will only be redressed when there are some tangible examples of how even a poorly-paid profession, such as research in Britain, can still bring rewards. □

Plague upon plague

The British decision to back research on slow virus diseases is prudent, not a sign of impending crisis.

BOVINE spongiform encephalopathy (BSE) is a disease of cattle apparently similar to scrapie, which afflicts sheep. There is a suspicion that scrapie may transfer to cows in the British farming practice of using unwanted offal from



one species as feed for another. The Southwood report to the British agriculture ministry last year valuably unravelled the likely origins of the disease among cattle, suggested measures (adopted by the government, which had no choice) to contain the spread of the disease among cattle, and left open the question of whether there are circumstances in which people might be similarly afflicted, possibly by consuming meat (or offal) from infected cattle. It is a chilling prospect, one certain to condition general approval of the decision of the British government and research councils to commission a package of studies bearing on the prevalence and causation of this and similar diseases (see page 196).

The question is the more chilling because the evidently infectious organisms responsible for scrapie and BSE are still obscure. It is bad enough for people's capacity to sleep the whole night through that there should be viruses, such as that responsible for AIDS, to which there is as yet no certain defence. What is to be made of an infectious agent yet to be characterized? It is no disrespect to Stanley Prusiner, of the University of California at San Francisco, and to his advocacy of the notion that the infectious agent is a piece of protein molecule called a 'prion' to suggest that speculation about the causation of disease can be the only guide while so little can be learned in the laboratory about prions and their function.

That is why it is important that the British government's decision to back research in the field should not be misinterpreted, or the significance of its decision exaggerated. The circumstantial evidence assembled by the Southwood committee for believing that cattle acquire their illness by being fed sheep offal is strong, for which reason the banning of that practice (if thoroughly enforced) should quickly bring the disease among cattle to a halt. But that is also the best way of fending off the chance that people might eventually be infected. The interdiction on the sale of infected cattle to abattoirs is likely to be less effective, not simply because there may be some farmers eager to sell afflicted cattle for more than the government pays in compensation, but because there is no way in which infected cattle not showing symptoms of disease can be spotted on the way to slaughter. Yet if Southwood is correct, and if the regulations now in force are effective, the epidemiologists (of cattle) soon to begin work should have the pleasure of recording a disease in decline.

It is also important that what may prove to be a passing scare about people's health should not be taken as yet another sign that there is something rotten at the heart of modern society — the original sin of the affluent. Even the practice of feeding processed sheep offal to cattle may legitimately be regarded as a means of economizing in natural resources that, in other circumstances, would be known by the green epithet of 'recycling'. That it has been discovered to be an unsafe practice (for cattle), at least when sheep are afflicted by scrapie, is fortunate rather than a cause for shame. The idea that people might ultimately be affected is a hypothesis only, but one that must not be put to the test. □

Religious strife

Communal unrest in Azerbaijan can be ended only if the Soviet Union hastens the process of economic reform.

MR Mikhail Gorbachev's latest trouble, in Azerbaijan, is serious and will not easily be resolved, either in Baku (the republic's capital on the Caspian) or in Moscow. In the south, the difficulty has been evident for at least two years, during which Armenians and their Azeri neighbours have been on tenterhooks about the Armenian enclave of Nagorno Karabakh, a mountainous region within Azerbaijan. Disappointingly, communal conflict was hardly interrupted by the damaging earthquake in Armenia just over a year ago, but it is far from clear why the Azeri population should apparently have taken up arms against Armenian residents of Azerbaijan so soon after prevailing on Moscow to end direct rule in the disputed territory. On the face of things, that looks like a serious miscalculation. Meanwhile, the big surprise — and the chief cause for everybody's alarm — is that so many of those living in the region appear to be able to put their hands on a rifle or some other kind of firearm.

Moscow has no choice but to restore order, whatever kind of settlement may be on the cards. Governments owe even minority populations no less. It is also unthinkable that the Soviet government should withdraw the troops now on the way to Azerbaijan without also disarming the local population, no simple matter. But there is no obvious way in which the *status quo* can be restored in Azerbaijan, for the underlying conflict is religious. The far south has become the Soviet Union's equivalent of the British government's difficulty in Ulster. That should be a proof, if one were needed, of how obdurate these problems are. And although Gorbachev promised, in Lithuania last week, that there would soon be a draft statute to regulate the ways in which individual republics would be allowed to distance themselves from the Soviet Union proper, while remaining part of a federation, he had little to say about the most difficult part of that exercise — the protection of minorities' rights.

In Moscow, the argument will be for still higher stakes. Gorbachev will not be able to deny that the trouble in the south would never have broken out had it not been for *glasnost* and what has flowed from that — the legitimization of dissent and of public protest in particular. There will be plenty of people ready with that complaint, to which the only true response is that conditions elsewhere in the Soviet Union would now be much worse if people were still prevented from saying what they think, as in the old days. The pity, for Gorbachev's cause, is that *perestroika* has so far lagged behind *glasnost*. As in Ulster, it is probably too late now to restore communal peace in Azerbaijan by the promise of prosperity to come. Only the reality of prosperity would do that, which is yet another reason for hastening the process of reform. Even Gorbachev's opponents must now acknowledge that. □

Japan's boom economy invests in science

- Second year of budget increase
- Space and nuclear research get priority

Tokyo

JAPAN'S Science and Technology Agency (STA) gets a big boost in its 1990 budget for research and development. Large increases go to space research, including study of the global environment, and to the internationalization of Japan's research system. But the agency, which funds much of Japan's government research outside the universities, has no plans to increase funding for research on the human genome despite recent US criticism of Japan's failure to contribute to the international project.

The budget was accepted by the Ministry of Finance and the cabinet at the end of last month and should take effect in April after approval by the Diet. But the opposition parties which now control the Upper House of the Diet are expected to gain a stronger foothold in the Lower House after next month's election. Passage of the budget could be delayed by a strengthened opposition, although outlays for science and technology are unlikely to be affected.

The agency's budget increases 6 per cent in its second year of expansion. Before 1989, budgets were held to increases at about the rate of inflation as the Ministry of Finance struggled to eliminate the issue of debt-financing bonds (a target that has now been achieved). The agency also won substantial extra funds in a supplementary budget drawn up at the end of last year to distribute larger-than-expected tax revenues from Japan's booming domestic economy.

The extra funds will be used in fiscal year 1990. One of the main beneficiaries will be space research and development, which in addition to winning a 10 per cent increase in the budget for fiscal year 1990 receives an extra ¥10,100 million (\$70 million), or about another 10 per cent, under the supplementary budget, according to Yoshiro Miki, director of the Policy Research Division of STA.

Most of the increases for space go to development of Japan's module for the US space station (¥9,796 million), the H-2 rocket (¥37,799 million), and the Advanced Earth Observing Satellite (ADEOS) (¥1,705 million). ADEOS will monitor the Earth's climate and ozone layer and is expected to be launched in the mid-1990s.

The agency's huge budget for nuclear energy research has begun to pick up again, after declining from 1986 to 1988. The experimental fast-breeder reactor

Monju, which is expected to reach criticality in 1992, gets a large slice of the funds (¥50,579 million), as does the JT-60 tokamak for nuclear fusion research (¥22,435 million). Outlays also increase for development of the world's largest synchrotron, the Synchrotron Orbital Radiation (SOR) facility. SOR will be located in Hyogo Prefecture near Osaka and is expected to be completed in 1995 at a cost of ¥100,000 million.

The *Mutsu*, a nuclear-powered ship that has not set sail under its own power since its reactor began to leak during its maiden voyage in 1974 (*Nature* 310, 531; 1984), gets another ¥3,689 million. The agency, which has spent about ¥110,000 million (about \$800 million) on *Mutsu* over the past 21 years, plans to send the ship on an experimental voyage this autumn before scrapping it. But it remains to be seen if Japan's powerful anti-nuclear movement will let the ship put to sea.

Outlays for ocean research decrease slightly because construction and sea trials of the deep-sea submersible *Shinkai 6500* have now been completed. But the budget includes ¥562 million for the development of a new unmanned vehicle for surveying the deep-sea floor down to 10,000 metres. Total construction costs for the vehicle are expected to be about ¥4,600 million.

The agency continues to expand the budgets for its small international research programme. ERATO will start four new projects in 1990 (as opposed to three in previous years). And the International

BUDGET FOR SCIENCE AND TECHNOLOGY AGENCY

	1987	1988	1989	1990	(% change from 1989)
	(thousand million yen)				
Total research & development budget	432.5	440.2	466.6	494.8	(+ 6.0)
Special Promotion Funds	8.4	9.2	10.1	10.2	(+ 1.0)
Space	94.6	98.5	109.1	119.4	(+ 9.5)
Nuclear energy	273.4	271.5	281.6	296.2	(+ 5.2)
SOR	0.07	0.6	1.9	2.8	(+47.4)
Ocean research	7.7	9.3	10.6	9.9	(- 6.6)
ERATO	3.2	3.8	4.6	5.1	(+12.4)
Human Frontier Science Programme	0.2	0.5	2.4	3.2*	(+33.8)

*Includes ¥ 1.3 thousand million from budget of Ministry of International Trade and Industry (MITI).

Frontier Research System, which is centred on the agency's Institute of Physical and Chemical Research (RIKEN) near Tokyo, will be expanded by establishing a new photodynamics research laboratory near Tohoku University in Sendai.

David Swinbanks

No special treatment for HUGO

Tokyo

THE Human Frontier Science Program, which opened in Strasbourg last year, continues to grow in the new budget. But the human genome project, at one time considered as a candidate for incorporation in Frontiers, will get no extra funds from the agency.

In the early 1980s, the agency began to invest about a million dollars a year in the development of automatic DNA-sequencing machines in a collaborative programme with industry. And STA is now directing about the same amount into projects at RIKEN to sequence a small yeast chromosome and to map and eventually sequence human chromosome 21. But Makoto Furunishi of STA's life science division says there are no plans to increase the budget for the RIKEN project, which gets ¥213 million in 1990.

Furunishi says that researchers interested in genome research can apply to the Human Frontier Science Program for

support. But the agency has no intention of contributing to the Human Genome Organization (HUGO) which will coordinate international research on the human genome. Furunishi says it is not clear why HUGO is necessary. High-energy physicists carry out big international projects without a central organization so "why do biologists need HUGO?", he asks.

Other government ministries are keen to launch human genome projects. Last year, the Ministry of Education, Science and Culture set up a task force under the leadership of Kenichi Matsubara, vice president of HUGO, to lay the groundwork for a project. The Ministry of Health and Welfare has just won about \$2 million in the budget for fiscal year 1990 to start a project to sequence human oncogenes. And the powerful Ministry of International Trade and Industry (MITI) is strongly rumoured to be preparing to launch its own technology-orientated project.

David Swinbanks

BSE causing public alarm

London

THE UK Department of Health is to back a study to follow changes in the incidence in Britain of Creutzfeld-Jakob disease, a rare but fatal neurodegenerative disease of man. The disease belongs to the family of spongiform encephalopathies which includes bovine spongiform encephalopathy or BSE, the so-called 'mad cow disease', an outbreak of which has excited great attention in the British press. There are fears, as yet unproven, that people eating meat from infected cows could go on to develop Creutzfeld-Jakob disease.

The first of several thousand cases of bovine spongiform encephalopathy was diagnosed in November 1986 after cows were fed meal containing tissue from sheep infected with scrapie, another spongiform encephalopathy. It is now generally accepted that the two diseases are caused by the same infective agent, but a link between them and Creutzfeld-Jakob disease is more controversial.

The official opinion of the Ministry of Agriculture Fisheries and Food (MAFF) is that the risk of transmission of bovine spongiform encephalopathy to humans is very small: "Scrapie . . . has existed in the national flock (of sheep) for more than 250 years without any evidence of human health problems", says Keith Meldrum, MAFF's chief veterinary officer.

Timothy Holt, of the Middlesbrough General Hospital, who has published a paper in the *British Medical Journal* on the possibility of a link between bovine spongiform encephalopathy and Creutzfeld-Jakob disease, acknowledges that the link is unproven, but he believes that the similarities between the two diseases have not received enough attention.

So far, there has been little success in isolating the infective agents causing spongiform encephalopathies. No specific nucleic acids have been found associated with the diseases, which is surprising if conventional viruses are responsible.

Some 10,000 cattle have been destroyed so far. Farmers receive only partial compensation.

One school of thought, championed by Stanley Prusiner of the University of California at San Francisco, argues that a protein called PrP is the infective agent, but his views are not widely shared. Researchers working at the Institute for Animal Health in Edinburgh have evidence that there are several different strains of scrapie, which suggests that some form of genetic material must be present.

It is in this climate of ignorance at the molecular level that the new epidemiological study is to be launched. Bob Will, of the Western General Hospital in Edinburgh, will lead the research. His team was involved with an earlier survey in England and Wales which collected data for the period 1970-85. The new survey will cover the whole of the UK and investigate the distribution of Creutzfeld-Jakob disease by place and occupation.

The study will be difficult to interpret as the disease has a long incubation period (from 18 months to more than 20 years). The researchers will need to look back at the previous life history of patients to find causative factors. Will says that his team will be looking for changes over time in the incidence of the disease. If there is a link with bovine spongiform encephalopathy, then an increase in the number of cases of Creutzfeld-Jakob disease should become apparent some time after the beginning of the cattle out-break.

The Department of Health's decision to support the study coincides with two separate announcements of additional funding for research into bovine spongiform encephalopathy itself. MAFF is to spend an extra £6.1 million on research on epidemiology and diagnosis over the next three years, in line with the recommendations of the Tyrrell committee report, published on 9 January. The Agricultural and Food Research Council will spend £6.3 million over the next three years, mainly on the molecular biology of the disease.

Peter Aldhous

Product licensing by phone

San Francisco

NORMALLY, a Product License Application (PLA) to the Food and Drug Administration (FDA) consists of 20 to 50 volumes, each 3 inches thick, of reports, charts, graphs and numbers detailing technical specifications and clinical results. But when biotechnology company Genentech last month filed a PLA seeking limited marketing approval for recombinant gamma interferon, it sent in instead a set of computer disks. The application is one of the first under a pilot study called CAPLAR (Computer Assisted PLA Review) being run by the FDA in conjunction with the Pharmaceutical Manufacturers Association.

The Genentech filing seeks permission to market interferon gamma-1b to patients with chronic granulomatous disease, an inherited disorder characterized by the failure of the body's white blood cells to kill invading bacteria. Although the filing is not yet complete, it is expected to take up about five standard diskettes, already configured for the FDA's software.

The submission marks a first for the CAPLAR programme and the FDA's Center for Biologics Evaluation and Research (CBER), which evaluates the vast majority of genetically engineered drugs. But a few new drug applications have been filed electronically under a similar programme called CANDAR.

Such submissions will not eliminate paper: reviewers still want paper copies to read. But FDA scientists will no longer have to wade through thickets of paperwork to find the chart or numbers they need to examine, and the data can be manipulated without numbers being manually transferred from tables to computers. "Reviewers can look at the data on disks and analyse the data on their own computers as they want to", said Kathryn Zoon, director of CBRE's Division of Cytokine Biology.

In CAPLAR's first trial, working out various kinks and bugs might take up more time than the usual procedure, she admitted. But she thinks the system will eventually speed things up.

Zoon said her division has also established an electronic mail link with Genentech to help mitigate the effects of the time difference between Washington and San Francisco and make it easier to get quick and complete answers to any questions that arise. Electronic filing is not a requirement for other companies, but she hopes it will become more common. "I think in the long run it will definitely make an impact on the way we do business", she said, adding that in the long run the new system will become necessary or "there'll be so much paper in the buildings that nobody will be able to move".

Robert Buder

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Panel endorses price rise

Washington

A PANEL of eminent physicists, assembled by the Department of Energy (DoE) to defend its Superconducting Super Collider (SSC) from cutbacks, weighed in last week with a strongly worded opinion that the accelerator should be built despite cost overruns that could reach nearly \$2,000 million.

Although the panel's conclusions come as little surprise (see *Nature* 343, 103, 1990), DoE officials have indicated that they believe such an endorsement from a influential advisory body (including five Nobel laureates) is necessary ammunition for the approaching congressional battle over the 1991 budget. Congressional critics have been saying that cost overruns are likely ever since the project began four years ago.

The panel was unequivocal in its support of the machine at its full design energy of 20 TeV. Lowering the beam energy to 17 TeV would "yield an insufficient cost reduction to justify the reduction in physics", the panel's draft report says. Below 15 TeV, the SSC would "unacceptably . . . risk missing important new physics". "The only way to keep the cost of the SSC at \$5.9 billion [DoE's original cost estimate] would be to seriously reduce its capabilities", the report continues. Asked to look for features that

could be initially removed for substantial savings, but could then be restored later, the panel said that it found none. One reason for not cutting the SSC's energy is that one quickly reaches a point of diminishing returns, Columbia University Nobel prizewinner T. D. Lee told DoE's High Energy Physics Advisory Panel (HEPAP). He explained that when looking for particles such as the Higgs boson, the desired 'foreground' signal roughly doubles in strength between 15 TeV and 20 TeV, while the background noise increases only linearly. Although there would be some cost savings by shaving the SSC's energy, the chance of exploring new physics would decline precipitously, he said.

Senator Phil Gramm (Republican, Texas), an SSC supporter in whose state the accelerator would be built, warned HEPAP panel members that the project was facing resistance in Congress, and that austerity would be necessary. "We are in a position of trying to compete for resources with other projects that have large constituencies", he said. "It is important that we end up not with a Cadillac, but a sturdy Chevrolet." Although he emphasized that he could not predict how far the SSC's cost could escalate before it reached its "political threshold", he said he could "try to get Congress over the sticker shock".

G. Christopher Anderson

FRENCH UNIVERSITIES

More for crowd control

Paris

FRENCH education minister Lionel Jospin has managed to secure an extra FF500 million (about \$80 million) from the government to offset overcrowding likely to provide a chaotic start to the new academic year. Next September, an extra 40,000 candidates are expected, all of whom have the right to a university place, having passed their *baccalauréat*. But, in Paris at least, most campuses are already severely overcrowded and building work under way will not solve the shortage of space in time.

The 1990 higher education budget, voted through parliament only a few weeks before Christmas, was already up by 9.5 per cent over 1989. But by the end of the year, it was clear that the FF27,500 million would not be enough. If the universities are already overcrowded, an estimated 200,000 square metres of teaching space needs to be found for the extra intake. Following a cabinet meeting on 10 January, it now seems certain that Jospin will receive the extra money he needs. Most of the new supplement will be used to rent space in addition to new buildings already under construction and due to open in the

autumn. A further 400 posts of lecturer will be created to cope with the new intake, and researchers will be paid to take on some lectures.

The extra money will relieve some of the stress in France's 71 universities and 66 technical universities. But additional libraries, restaurants and student accommodation are also required. Plans are under way to build five new campuses and to modernize what some claim is Europe's oldest university, the Paris Sorbonne. But this will take time.

In order to speed up the process, the government is considering incentives to regional and local governments willing to share the financial load. As universities can bring economic returns in the form of local investment — particularly when science and technology skills are available — many regions may be keen to accept. This is particularly likely for the depressed Nord region where one new campus is planned. A former mining area, the region is just at the entrance to the Channel tunnel, scheduled to open in the mid-1990s and a good site for a new European campus to rival Grenoble and Strasbourg.

Peter Coles

Weapons clean up

Boston

THE US Department of Energy announced last week that for the first time ever it would undertake sweeping environmental studies of its nuclear weapons production facilities. One of the 'programmatic environmental impact statements' will cover the department's plans to conduct a massive clean up of its existing facilities; another will review the agency's options in its efforts to modernize nuclear weapons production facilities.

The Energy Department's announcement came in the face of a lawsuit brought by eighteen environmental groups including the Natural Resources Defence Council. Although the Energy Department included no timetable or budget for the environmental reviews, it nonetheless marks a major step in opening up to greater public debate the potential environmental and health dangers from nuclear weapons production. S.S.

Out damned SPOT

Paris

A LAST-MINUTE failure in one of the tape recorders on board the French Spot-2 commercial Earth observation satellite has set the launch back by several weeks. According to a French national space research centre (CNES) communiqué, the recorder did not rewind under remote control during a final check while the satellite was already aboard its Ariane launcher on the pad at Kourou, French Guyana.

The tape recorder is one of the two on board that temporarily store images while the satellite is out of range of ground receiving stations. Two similar tape recorders from the same manufacturer, the US company Odetics, were installed on Spot-1. One of these stopped working soon after the satellite was put into orbit, leaving a few permanent blind spots. But the surviving recorder has continued to function for the past four years — twice as long as planned.

Spot-2 has now been removed from the rocket's payload area so that the faulty component can be replaced — an operation which could take more than two weeks. P.C.

Benveniste all-clear

Paris

Dr Jacques Benveniste, whose controversial work on the biological activity of high-dilution solutions earned him a reprimand from the French medical and health research institute (INSERM) where he works, will be allowed to continue as head of his laboratory until 1992, when his present contract expires. When Benveniste's research was examined in a routine review last year, Philippe Lazar, INSERM's director general, made the continuation of Benveniste's post contingent on 'good behaviour' — which meant keeping out of the press. P.C.

California set to get tough

San Francisco

REGULAR reviews of the performance of all tenured professors and a mandatory 'rehabilitation' programme for those found wanting are likely to be adopted by the University of California (UC).

The new measures, a compromise after a more radical proposal was defeated in a vote last year, will signal the end of protection of incompetent faculty members within the nine-campus UC system and will almost certainly lead to changes at other universities.

In May 1986, the University Committee on Academic Personnel (UCAP) urged each campus to adopt a policy of mandatory review for all professors at least every five years. The University of California at present has an eight-step pay scale for tenured full professors, with no mandatory reviews after the fifth level is reached.

The Berkeley campus adopted mandatory review the following September and then went a step further, examining what could be done when the review shows inadequacy in both teaching and research. Pressure from legislators, who believe that money could be saved if ageing senior faculty were replaced with more junior members, was one factor stimulating action. Another was the 1986 amendment to the Age Discrimination in Employment Act which by 1994 will eliminate mandatory retirement for university faculty and could allow faculty to stay at work well into their dotage.

By 1988, Berkeley had come up with a set of recommendations which suggested that when both teaching and scholarship standards became dismal, appointments should be terminated. But automatic dismissal was judged too severe when presented to other UC campuses, and last June a university committee was set up to work out measures acceptable to all. Its conclusions are now becoming clear, although it is not expected to deliver a preliminary report until the end of the academic year.

The committee chairman, chemistry professor Charles Nash of UC Davis, says that the Berkeley proposal read like a "search and destroy mission". He favours instead regular reviews for all faculty. In the course of these evaluations, he said, "there might be identified an occasional individual for whom additional inquiry might be appropriate". Nash said the new plan will probably enhance standing rules that suggest rehabilitative procedures for failing faculty. "My gut feeling . . . would be simply to mandate rehabilitative efforts as a first step", said Nash. The existing policy of terminating a faculty member only if rehabilitative efforts fail would not change.

Favouring the more low-key approach

is the American Association of University Professors, which feels that most universities already have adequate safeguards against faculty incompetence. "It's almost standard for a university to include a lack of competence as one of three general categories that are grounds for dismissal", said Jordan Kurland, its associate general secretary. The others are misconduct and neglect of duties.

One problem is that despite their rules, few schools have ever done anything

ACADEMIC TENURE CONFIDENTIALITY

Discrimination case victory

Washington

US universities will have to reappraise the need for confidentiality in tenure review following a Supreme Court ruling that federal investigators can force universities to show them confidential tenure evaluations. The court decided in favour of the Federal Equal Employment Opportunity Commission (EEOC) and Rosalie Tung, a former management professor at the University of Pennsylvania. Tung, a Chinese-American, had complained that she had been unfairly denied tenure because of her sex, race and national origin.

The court rejected the university's argument that removing a guarantee of anonymity would discourage "candor" in tenure decisions. "Not all academics will hesitate to stand up and be counted when they evaluate their peers", Justice Harry Blackmon said in a written opinion.

The case is seen as critical by many US universities. "Our concern is that eliminating confidentiality will drive the tenure process underground", says David Merkowitz, director of public affairs for the American Council on Education. He believes that the knowledge that a reviewer's name can be handed over to federal investigators, and aggrieved tenure candidates, could have a "chilling effect" on the review process. Rather than submitting written and signed opinions, peer reviewers may be willing only to discuss the issue in private. And then, Merkowitz says, the "old boy network will be making the decision". The immediate reaction in the academic community seems to be a cautious wait-and-see. At the University of Pennsylvania, general counsel Shelley Green says that no immediate changes to the tenure process are planned. "If we find that there are problems in getting recommendation letters [from peer reviewers], we'll have to reappraise our policies", she says.

One likely compromise, at least for the immediate future, is for universities to hand over review documents but to withhold the names of the reviewers. That

about incompetent faculty members. And any step by the University of California system toward changing that situation is likely to affect the approximately 5,000 four-year institutions in the United States and their 200,000 tenured faculty members, said Kurland. "The University of California is basically an enlightened system, a terribly influential system, and whatever they do is important", he says.

Nash agreed, citing a new era for universities. "The idea that the demonstrably incompetent tenured faculty member is protected, that's what is dead", he said.

Robert Buder

method is already practised by the National Science Foundation and the Department of Health and Human Services (HHS) in their grant-approval process, as well as by many scientific journals in reviewing manuscripts submitted for publication. In two separate cases last year, HHS's Centers for Disease Control and the journal *Physical Review Letters* both survived lawsuits that sought to reveal the names and comments of peer-reviewers. And at the National Institutes of Health, individual comments are destroyed after they are summarized, effectively ruling out a subpoena.

But the tenure process may not be so resistant to lawsuits. "The courts have to balance the need for academic freedom with Congress's dictate of non-discrimination in employment", says Allan Ryan of Harvard University's general counsel's office. And he suggests that the will of Congress may often take priority.

Although Harvard recently won a decision which allowed it to obscure reviewers' names in a tenure decision, that case is under appeal.

Future suits are likely to be settled on a case-by-case basis, says Thomas Wright, general counsel for Princeton University. If the investigators can convince a judge that the selection of reviewers may have been inappropriate, the university could be forced to reveal their names, he says. Nevertheless, until some legal precedent is set, Princeton and several other prominent universities have said that they intend to turn over peer-review documents without the names of the reviewers and fight the court battles when they come.

G. Christopher Anderson

Tunnel still news

EUROTUNNEL, the company managing the present attempt to link England and France, last week secured finance to keep the Channel tunnel alive. Was the optimism displayed by *Nature* 120 years ago justified? See Then and Now, facing the inside back cover.

UK SCIENCE BUDGET

Environment benefits in UK

London

THE Natural Environmental Research Council (NERC) and the Agricultural and Food Research Council (AFRC) come off best in the shareout of the UK science budget released last week by John MacGregor, Secretary of State for Education and Science. Both receive about 10 per cent more than in 1989–90. MacGregor stressed that the allocations to the five research councils will benefit environmental research.

Funds are set aside for instruments to be carried on the European Space Agency's new remote-sensing satellite (ERS-2), and for NERC to participate in the World Ocean Circulation Experiment, results from which will help scientists model global climate change.

In distributing the total £897 million (up from £824 million in 1989–90), the secretary of state stuck, almost to the letter, to advice he received from the Advisory Board for the Research Councils (ABRC). The ABRC advice, published to coincide with MacGregor's announcement, also recommends allocations for the two following years, based on the government's planning figures for science spending. ABRC warns that the government's figures imply "some reduction in the amount of science that can be supported by 1992–93".

NERC's increased share of the budget includes over £17 million for the British Antarctic Survey's new research ship, the *James Clark Ross*. But nearly half of this is unspent money from 1989–90, carried over into the present budget.

AFRC will spend £12 million over the next three years continuing the restructuring of its research institutes onto fewer sites. Professor Bill Stewart, Secretary of AFRC, is pleased that the end of restruc-

THE 1990–91 BUDGETS FOR
UK RESEARCH COUNCILS

	£ million
Agricultural and Food	85.91
Economic and Social	36.01
Medical	185.71
Natural Environment	135.23
Science and Engineering	438.62

Smaller amounts went to the Royal Society and ABRC.

turing is now in sight so that, "council will be able to concentrate its funding in future on new science".

With the exception of the Medical Research Council (MRC), which fears that it must withhold funds from highly rated research projects, the councils seem satis-

UK UNIVERSITIES

Dual-support system to go?

London

MAJOR changes in the way UK scientific research is supported in the universities are recommended in a consultative paper from the Department of Education and Science. The aim is to shift responsibility for funding the overheads of research from the universities to the research councils.

Under the present 'dual support' system, the research councils provide about £250 million a year to pay for additional costs associated with projects they sponsor, but the universities, through their block grant from the Universities Funding Council (UFC), pay for existing staff and facilities. The problem is that this distinction has become increasingly blurred, with considerable argument over the balance of funding for some projects.

fied with their allocations. AFRC regards the news as "excellent and encouraging", while the Science and Engineering Research Council (SERC) talks of "a reasonable settlement".

Commenting on the varied financial fortunes of the research councils for 1990–91, science minister Robert Jackson said that a true picture emerged only if funding was considered over a number of years. A ten-year survey showed that the proportion of funds received by each council varied very little, compared with support for different subject areas within each council. This indicated strong management within councils, but too little co-ordination between them. It is these considerations that prompted ABRC's Morris report to recommend amalgamation of the councils, said Jackson. A decision on the recommendations is expected "very soon". **Peter Aldhous**

If the government's scheme is introduced, from 1991–92 some £70 million per year will be transferred from the UFC grant to the research councils' budgets. Applications for research grants must give accurate costings for overheads, such as equipment maintenance and computing costs, to be funded by the research councils. A standard percentage will be added for other, less identifiable expenses, such as telephone charges and library costs.

But there are fears that research supported by charities, which support more medical research in the universities than do the research councils, could suffer under the new arrangements.

Research supported by charity currently benefits from the UFC block grant in much the same way as research council projects.

Although the consultation paper notes that the government does not wish to see any reduction in indirect support for the charities' projects, the Association of Medical Research Charities is worried that there is no explicit mention of a mechanism to maintain the payment of overheads from UFC money.

If universities started to impose charges on the charities for overheads, this "would result in a reduction in the quantity of funded research".

The heads of UK universities are also considering the new system. A spokesman for the Committee of Vice-Chancellors and Principals said that an initial concern was the continued existence of UFC funds to sponsor research from promising new scientists, without the proven 'track record' to attract research council money. The committee is anxious that this facility should continue, despite the reduction in the UFC grant. **Peter Aldhous**

JOURNAL CITATIONS

Japanese papers top the charts

Washington

JAPAN may complain about the low standard of its basic research but journal citation figures tell a different story. For the second half of 1989, Japanese groups take two out of the top three positions in the lists of most cited papers in both biology and physics — the first time that this has happened.

The figures come in a new monthly journal called *ScienceWatch* published by the Institute for Scientific Information, the publishers of *Current Contents*. In positions two and three on the biology list are papers on endothelin from T. Masaki *et al.* of the University of Tokyo and on protein kinase C from Y. Nishizuka of Kobe Medical School. An earlier paper by Nishizuka

broke records by becoming the most cited paper of the 1980s. This paper and the two others were all published in *Nature*.

At the top of the physics list is a paper by T. Asano *et al.* of the National Research Institute of Metals on a high-temperature superconductor, published in the *Japanese Journal of Applied Physics*. In the number three position comes S. Uchida *et al.*'s paper, from the University of Tokyo, on a superconducting copper oxide compound, again published in *Nature*.

But while Japan can now rightly claim some pinnacles of excellence, the United States still owns most of the high ground.

All the remaining 16 papers in the two top ten lists come from US laboratories.

Alun Anderson

Hacker trial under way

Washington

THE trial of Robert Morris Jr, the college student charged with creating the 'Cornell virus' that struck thousands of US computers in 1988, began last week with only one thing clear: there seems little doubt that Morris is responsible for the rogue program.

What is far less certain is how current law should be interpreted in the case and what, if anything, a conviction will change.

Although most experts say there is compelling evidence that Morris wrote and released the computer program, many expressed surprise that the government had decided to prosecute him.

The law under which Morris is being prosecuted, the previously untested 1986 Computer Fraud and Abuse Act, is a

graduate student at Cornell, in New York, but he first released the virus by sending it to a machine at Harvard University in Massachusetts. And among the computers subsequently infected by the virus were dozens of machines at government laboratories.

What remains to be seen is whether Morris can be said to have 'accessed' the infected computers himself. No doubt the virus did eventually break and enter many machines, but by the time it began reproducing and transmitting itself across the country, Morris was just another bystander, helpless to stop it.

The very fact that prosecutors are forced to wrestle with such semantic questions is a reminder of the inadequacy of the current law, congressional aides say. They say that a more workable statute would focus on questions of criminal intent and malice, and define terms such as 'access' in such a way that direct agents such as virus are obviously included.

At least four bills to revamp the laws on computer use have been introduced in Congress, but even their supporters acknowledge that none of them would completely solve the problem. "It's a real challenge", says one Judiciary Committee staff member. "You have to anticipate the way people may be using computers in the future." Using specific terminology such as "virus" and "worm" is one pitfall that should be avoided. In 1986, when the current law was written, the very concept of a virus was known only to a small computing underground. And even today's hackers are hard pressed to guess what the computer crime vehicle of the future will be, or by what new name it will be called.

Morris has pleaded not guilty to the charges against him. His lawyers argue that he showed no malicious intent, and was trying only to demonstrate the weaknesses of the nationwide network. The virus did no permanent damage and only slowed the infected computers as it reproduced itself.

If Morris is found innocent of the charges, then the 1986 act has failed in its first test, and is badly in need of an overhaul, according to a Senate Judiciary Committee staff member. Such a decision would no doubt spur Congress to pass new legislation promptly. And even in the more likely case that he is found guilty, a congressional reappraisal of the whole issue of computer crime seems likely. But lawmakers are wary of overreacting by making the law so draconian that mere sloppy programming is against the law. "We have to use a scalpel, not a club", says one aide. "A bad bill can do more harm than a virus."

G. Christopher Anderson

Photographed in 1988, Cornell graduate Robert Morris Junior.

makeshift statute aimed as much at preventing bank fraud as at controlling network mischief. To avoid infringing on the states' rights to create their own legislation, the law confines its scope only to those crimes committed on 'federal interest computers' — a largely artificial distinction that has little bearing on computer crime, experts say. The law requires that a suspect must have either illegally accessed a government computer or used computers in two or more states in committing the offence.

According to the Justice Department indictment and an investigation carried out by Cornell University last year, Morris violated both parts of the statute. He was a

Pollution clean up promised

Washington

IN reaction to charges that US Antarctic bases are polluting the environment, the National Science Foundation (NSF) last week issued a pair of reports on how it could bring its polar activities into "full compliance with all applicable laws and foster environmentally responsible conduct by United States nationals in Antarctica".

NSF activities in Antarctica first came up public fire in 1988 when the Washington-based Environmental Defence Fund claimed that McMurdo Sound was more heavily polluted than the United States own coastal waters (see *Nature* 334, 643; 25 August 1988). McMurdo Station is the largest base in Antarctica with more than 1,000 scientists and maintenance crew in residence in the summer. The two other US bases are Amundsen-Scott at the South Pole and Palmer at Anvers Island off the Antarctic peninsula. NSF manages the entire US Antarctic programme and has admitted that mistakes in managing pollution were made in the past. Recently, several tens of thousands of gallons of fuel leaked onto the ice at McMurdo station.

The new plan, plus approval by Congress to spend \$30 million over the next four years, provides for the removal of all dangerous wastes from the bases, new equipment to clean up fuel spills, waste treatment plants at McMurdo and Palmer Stations, and a clean up of abandoned field camps. NSF will also develop a comprehensive programme to govern discharge of all pollutants.

Alun Anderson

SPACE MANOEUVRES

Dexterous performance by shuttle crew

Washington

ASTRONAUTS on the US Space Shuttle Columbia successfully recovered the Long Duration Exposure Facility last Friday, manoeuvring the 11 ton orbiting laboratory into the shuttle cargo bay with the help of the Canadian-designed 50 foot manipulator arm.

The Long Duration Exposure facility has been orbiting the Earth for five and a half years. Once back on the ground, scientists will be able to assess experiments that include those designed to determine the long-term durability of structures placed in space (see *Nature* 343, 847; 21/28 December 1989). Photographs taken of the facility before it was placed in the cargo bay show that the metal foil covering some panels is damaged, peeled back or missing altogether. Columbia is expected to return to Earth on Friday this week. The ten-day flight, double the length of most shuttle missions, was partly designed to test future plans for month-long flights.

Alun Anderson

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Losses no worry to Boston

Boston

BOSTON University's controversial investment in a small biotechnology company called Seragen Inc. is drawing new criticism as reports surface that the university will write off another \$16 million of its sizeable stake in the company. The latest revelation means that the total losses written off by the university are now greater than the initial \$25 million the university spent to acquire a majority interest in the company in 1987.

With no immediate prospect of products or revenue from the fledgling company and with Boston University covering the company's entire operating costs in the form of 'loans' totalling approximately \$1 million a month, the Boston University—Seragen situation is a financial arrangement without precedent or peer in the realm of academic investment. \$60 million in university funds has been invested in the company since 1987—a sum equal to nearly a third of the university's endowment.

But university representatives call the recent write-off merely a prudent, "conservative accounting procedure", and add that they remain confident that their investment will ultimately pay off. Boston University's controversial president, John Silber, widely perceived to have staked his reputation on the investment, has repeatedly defended the company's prospects, calling it a "future Xerox", and predicting revenues of billions within the decade.

Nonetheless, many Boston University faculty members, including the retired dean of the university's business school, have publicly called the arrangement "crazy" and "irresponsible" and accused Silber and other university administrators of siphoning off much-needed university dollars to support a risky commercial venture.

What makes the situation so extraordinary is not only the relative size of such a highly speculative investment, but the fact that Boston University, as 70 per cent owner of the company, has committed itself to supporting the firm indefinitely out of 'discretionary' university funds. The university has, in effect, taken full financial responsibility for a commercial venture—one that currently employs 100 people. And even if it does succeed, may not turn a profit for many years.

Although a risky investment for a university, Seragen's research aims are considered it sound. The company has patented a method to produce hybrid proteins using a 'tamed' diphtheria toxin. The hybrid proteins—so-called chimaeric receptor toxins—are designed to target particular receptor cells for the treatment of autoimmune diseases, such as certain forms of cancer, while leaving other cells

in the body unharmed.

Seragen has pioneered the use of the diphtheria toxin, but the more general research strategy has attracted the attention of many companies.

Thasia Woodworth, Seragen's clinical and regulatory affairs director, stresses that the company has products in "every phase of the pipeline", but only one potential product is currently undergoing clinical trials in humans. The drug, called Leukocytotoxin-L, is designed to treat adult T-cell leukaemia by pairing the altered diphtheria toxin with the interleukin-2 binding site. The result, Seragen hopes, is a protein that will attack and kill altered lymphocytes. Related research at the company employs the hybrid molecule technology to bring the altered diphtheria toxin to kill cells causing malignant melanoma, and those implicated in the rejection of transplanted organs.

The Seragen investment renews questions about the extent to which a university ought to support the commercialization of research.

Seragen's connections with Boston University are said to date back to the early 1980s when a small company was founded with funds from the university's venture capital fund. The controversial part of Boston University's investment, however, began in 1987 when, apparently at Silber's forceful urging, the school acquired a majority stake in the company from a Norwegian pharmaceutical company wishing to sell off its investment in Seragen. Boston University's purchase came just two months before the stock market crash in which biotechnology companies were particularly badly hit.

At least as controversial as the Seragen investment is Silber himself, who is now unofficially considered a conservative democratic candidate in the upcoming gubernatorial race in Massachusetts. Silber's caustic and authoritarian style has earned him many bitter enemies inside and outside the university. Nonetheless, he is credited with many achievements during his 18-year tenure as president of Boston University. Unquestionably, the school was in poor shape when he took over. A chronic operating deficit was quickly corrected, and the school's endowment, which in 1970 stood at a mere \$19 million, has since grown nine-fold. Silber even managed to attract both US President Bush and French President François Mitterrand to speak at the university's 150th anniversary celebrations.

Yet even some Silber supporters remain highly sceptical of the Seragen deal. And official university justifications of the arrangement have been criticized as too defensive and farfetched. University

vice president John Westling, for instance, says adamantly that "no endowment funds have ever been used in any of the transactions with Seragen". But critics, such as Rich Cowan, speaking for the National Coalition for Universities in the Public Interest, counter that the distinction is largely semantic: the 'discretionary funds' used in the Seragen investment, they say, could have been used to increase the school's endowment by 30 per cent. "Whether technically endowment funds or not", says Cowan, "the school could have endowed five or six new faculty positions instead of funding a commercial venture." But Westling defends the Seragen investment as an example to university researchers that the school "actively supports them in bringing the fruits of their research to market". Without arrangements such as the Seragen investment, Westling contends, universities in the United States will "lose the ability to attract and sustain top researchers in the academy", a prospect he says would deliver an "extremely damaging blow" to the nation's scientific enterprise.

Entrepreneurial wizardry or financial folly, the Seragen arrangement will remain controversial. Despite growing fears that the university is like a drug addict with a "\$30,000-a-day biotech habit", as one critic has put it, university officials such as Westling and Silber remain unshakeable in their devotion to the biotechnology company. Controversial or not, Westling states the university position unequivocally: "As it stands now", he says, "we have no intention of getting out."

Seth Shulman

AUSTRALIA

New CSIRO man to stress industrial links

Sydney

JOHN Stocker has been named as the new chief executive of Australia's Commonwealth Scientific and Industrial Research Organisation (CSIRO). Stocker is at present managing director of the Australian Medical Research and Development Corporation (AMRAD) and will take over from Keith Boardman on 5 March 1990.

Stocker's background is in industry, fitting in well with CSIRO's conviction that science must be made profitable. "CSIRO's biggest problem is in its dialogue with industry", Stocker says, "Scientists, until now, have had difficulty in convincing industry of the relevance of science." Stocker is also concerned with motivating CSIRO scientists and says he will be "looking at the introduction of performance related awards".

Tania Ewing

■ Australia features in this week's Commentary, page 203.

Time for change in taxonomy

SIR—Biologists repeatedly complain about instability in classification and taxonomists repeatedly reply that their work aims at greater stability. For example, Hawksworth¹, a taxonomist, agrees with Crisp and Fogg² that name changes for taxonomic reasons are tiresome. We think the complaints are unjustified and that the quest for stability is mistaken. Change is an essential quality of taxonomy, which is a part of science, as pointed out by van Valen³ and Newman⁴.

There is good work and bad work in taxonomy as in all branches of science, but changes of classification are not always simply consequences of nomenclatural juggle or "mere changes of opinion"⁵. Classification is the means by which taxonomists describe scientific progress. Proposals to freeze renaming for periods of several years, as suggested by Barnett⁵, or to establish some international peer-review system to approve or reject proposed modifications^{1,5} overlook the benefits of change in taxonomy. Our present taxonomic system is burdened with a plethora of unnatural taxa conflicting with what is known about evolution and phylogeny. Taxonomists work at removing these offending elements, which are a persistent source of error in much biological research. In order to improve their own research, biologists should check the latest and best classifications of the groups they study and welcome improvements in

their reference system, rather than object to change for mere convenience.

The problem with taxonomic instability is not one of science, but of information. There is a vast amount of taxonomic information about the many organisms involved. It is difficult to keep track of all name shifts. But those are meant to improve our understanding of relationships, and we do not need means of retarding that process. Instead, we need international database systems carrying continuously updated taxonomic information and providing easy access to synonymy and recent literature.

Taxonomists should pursue their scientific venture and stop worrying about instability in classification. Taxonomy is not a service function for labelling organisms, but a science of its own, dealing with variation, relationships and phylogeny. Other biologists need to keep themselves informed, and should realize that removal of artificial groups and improvements in classification are desirable.

KÅRE BREMER

Uppsala University,
Department of
Systematic Botany, Box 541,
S-751 21 Uppsala, Sweden

BIRGITTA BREMER

University of Stockholm,
Department of Botany,
S-106 91 Stockholm, Sweden

PER OLA KARIS

MARI KÄLLERSJÖ

Swedish Museum of Natural History,
Department of Phanerogamic Botany,
S-104 05 Stockholm, Sweden

1. Hawksworth, D.L. *Nature* **337**, 416 (1989).
2. Crisp, D.J. & Fogg, D.E. *Nature* **335**, 120–121 (1988).
3. van Valen, L.M. *Nature* **323**, 664 (1986).
4. Newman, W.A. *Nature* **337**, 23–24 (1989).
5. Barnett, J.A. *Nature* **338**, 547 (1989).

Letter from Japan

SIR—Recent articles have addressed the opportunities for foreign scientists in Japan and asked why they are not very eagerly taken up (*Nature* **340**, 337 & 339; 1989). Differences in culture and problems with language have been suggested as part of the reason. Although some of the exchange schemes mentioned do offer funds to pay for language classes, after a day's work in the laboratory many may not have the time or energy to devote to a difficult language. A disincentive to study is the time required to reach a level where steady improvement is possible through everyday use.

I am part of a European Commission (EC) scheme to support European scientists for 12–18 months research in Japan. An important element of this programme is a three-month intensive language

course as a group in Tokyo, before starting research work. This not only teaches the basics of spoken and written Japanese, but also provides an opportunity to learn about Japanese culture and build contacts with other European scientists in Japan. As a group, we keep in touch through newsletters and meetings throughout our stay here and afterwards.

Hardened researchers may question if three whole months away from the laboratory is justified. But many months can sometimes be spent in research that leads nowhere. It is a question of making an investment and if one is not prepared to do so, perhaps one should not be thinking about coming to Japan. There is a new enthusiasm for basic science here, new money and beneficial ways of looking at problems and for solutions, which it may be beneficial for the visitor to learn. But understanding can come only through contact and participation. And who knows what inspirations may appear while learning to write the *Kanji*?

T. J. P. HUBBARD

(Visiting Research Fellow,

European Community

Scientific Training Programme)

Protein Engineering Research Institute,
6–2–3, Furuedai, Suita,
Osaka, 565 Japan

■ Further information about the EC scheme may be obtained from: Mr M Merla, DG XII-G-3, Commission of the European Community, Rue de la Loi 200, B-1049 Brussels, Belgium.

AIDS in Canada

SIR—My dismay at hearing that Prime Minister Margaret Thatcher (*Nature* **341**, 181; 1989) had turned down a proposal to carry out a national survey of people's sexual behaviour to help to understand better the spread of AIDS through sexual transmission of human immunodeficiency virus (HIV) has now been tempered by the decision of the Wellcome Trust to support the proposal with a grant of £900,000.

We in Canada have faced a similar situation since our government has not responded much to the many research funding recommendations submitted, in April 1988, through the Royal Society of Canada's sponsored report entitled *AIDS, A Perspective for Canadians* (see *Nature* **335**, 2; 1988). We are still concerned that our country will fall behind in its fight against this deadly disease. Canada is a rich country which should make a contribution to the world pool of knowledge in order to help the hundred of thousands patients affected by AIDS.

MICHEL CHRÉTIEN

Institut de Recherches Cliniques de
Montréal (ICRM),
Montréal, Canada H2W 1R7

Fishy tale

SIR—Peter Newmark's otherwise limp exposition of themes developed at the recent *Nature* conference on Gene Manipulation in Biology and Human Disease (*Nature* **342**, 221; 1989) was rendered somewhat confusing by his statement that "transplantation is a type of gene therapy . . . that cocks a snook at the sophistication of genetic engineering". Cocking a snook is a sophisticated achievement, indeed, if related to the transfer of genes (presumably from the Y chromosome) of a chicken, to "any of a family (Centropomidae) of percoid fishes of warm seas; esp. a large game and food fish (*Centropomus undecimalis*) of the tropical Atlantic". One initially imagines that the reasoning behind this experiment is that if the fish started to crow they would be easier to catch. It is hardly sporting of Newmark to give the bird to the fraction (presumably the majority) of *Nature*'s readers unprepared for such a uniquely British idiom.

LEE LESERMAN

Centre d'Immunologie de
Marseille-Luminy, Case 906,
13288 Marseille Cedex 9, France

The Dawkinsization of Australia

John Maddox

Australia's universities begin this month an academic year that will see them well on the way towards a unified system of higher education. Hopes that an election later in the year will bring a change of course, probably misplaced, are unlikely to be fulfilled. But nobody can guess what the consequences will be except that they will be profound.

Sydney (December)

To many Australian academics and researchers, the passing of 1989 will have brought a great sense of relief. For the past year, and longer, the university system has been confronted with a radical reorganization plan whose consequences must be profound. During the same year, it became plain that the Canberra government seriously intends to carry through its plan for concentrating research support on a small proportion of the nominally eligible researchers. Meanwhile, the future of the Commonwealth Scientific and Industrial Research Organization (CSIRO) is again uncertain, despite the seven formal attempts to define its role during the past few years, as the federal government has sought a chairman to succeed Dr Keith Boardman, who retires later this year. Its appointment of Dr John Stocker to the post (see this issue, page 201) will inevitably create a sense that CSIRO's distinctive culture may now be changed.

Those who look back on 1989 with disdain look forward to the year ahead with enthusiasm, not least because the Hawke Labor government must call an election, most probably in the austral autumn. Many in academic life openly rub their hands with pleasure at the many signs of the government's discomfiture — the financial failure of Australian's generation of international entrepreneurs spawned during the 1980s, the continuing problems of the national economy and even the embarrassment of the middle-class strike by the pilots of the domestic airlines (which, among other things, has damaged the balance of external payments by keeping tourists away from Australia). The hope is that, on grounds such as these, Mr Bob Hawke and his colleagues in government will be turned out a few months from now, and that the painful reorganization now under way will be halted, perhaps even reversed.

But the calculation could easily be wrong. The impending demise of the Labor government would be a more certain outcome if the political opposition to the Canberra government could manage to present a more coherent policy. In their sense of adversity, embattled academics also overlook the advantages that accrue to governments already in office. Moreover, there can be no guarantee that a new government would promptly abandon the

changes that Hawke and his ministers have set in train. Nobody should be surprised if 1990 turns out to be an extrapolation of 1989 into an unfamiliar future.

It is also relevant and important that the academic and research community is not at one in its opposition to the changes under way. Many, people as well as institutions, will benefit from what is proposed. Successful researchers can look forward to more generous support in future. Institutions newly transformed from technical colleges into universities have at least prestige to gain. And it is hard to quarrel with the government's chief objectives — to increase the proportion of young Australians benefiting from higher education and to concentrate research support on groups likely to be productive. Nor is it possible to cavil at the government's willingness to back its ambitions with resources.

So what explains the vehemence with which recalcitrant academics and researchers denounce the Hawke government's plans? In these circles, one is quickly persuaded either that Mr John S. Dawkins, the Minister for Employment, Education and Training, is the least popular man in Australia, or that that dubious distinction lies with Professor Don Aitkin, the political scientist seconded from the Australian National University at Canberra to be chairman of the Australian Research Council, the rapidly growing source of research support for general research projects. Both men are able, and probably also ambitious. Why should their names so often excite invective and scorn — much of it in public — from people much more used to genteel differences of opinion?

It is a remarkable phenomenon, unlikely to be quickly exorcised, which is most easily accounted for by the practice of conviction politics, the phrase widely used to describe at least the early years of Mrs Margaret Thatcher's British government. The Dawkinsization of Australian higher education stems from Dawkins' evident conviction that his reforms are self-evidently in the national interest. Much of the abounding discontent stems from the dissenters' sense that he will not listen.

But the special tone of the Australian argument about the future of higher education and research derives from the extent to which Australia is an over-

governed democracy. There are probably more elected politicians per 100,000 population in Australia than anywhere, but the balance between the six elected state governments (and the two 'territories', the Northern Territory and the Australian Capital Territory) is also more delicate than in most other federal systems. In many fields, the Canberra government can get its way only by persuasion, to which end the spending of central funds is a common ingredient. Only occasionally, as now on higher education and research, does the federal government sense that its powers to act are relatively unfettered by states' unwillingness to comply with central plans.

Now, as always during the past 40 years, the federal government is also preoccupied with Australia's uncertain place in the modern world. Twenty years ago, Australians were preoccupied with the question whether an economy still then dependent on the export of the primary products of agriculture and minerals extraction could hope to keep up with industrialized economies adding value to Australian raw materials. Why not export finished textiles, not wool, was one cry? Or enriched uranium, not just uranium oxide?

From that period and before sprang the complicated system by which Australia sought to protect domestic industries making products as different as motor cars (often from imported parts) and electronic goods. Now, the core of the argument centres on South-East Asia, called east Asia. May not the future lie in trading relationships with Japan, for example? (Australians have discovered the benefits of being in essentially the same time-zone, if in another hemisphere.) And may not the best strategy for the future be to dismantle the complicated systems of restrictions on the foreign ownership of Australian assets and even the import duties devised for the protection of industries whose promise has dimmed with the passage of time?

The prospects of closer relationships with — or more fierce competition from — Asia to the north is relevant to the Dawkinsization of Australian higher education for the simple reason that Australian participation in higher education is much less than in countries such as Japan. Dawkins' plan to create a unified system of higher education in Australia is in one

sense the fulfilment of the dream of Mr Barry Jones, Minister of Science in the Hawke government since its beginning seven years ago, whose evangelism in the cause of an educated Australia long antedates Hawke's election.

Unification

Those who compare the present upheaval in Australian higher education with what has happened in Britain over the past decade are only superficially correct. It is true, of course, that there are many similarities. Some academic institutions are being compelled to merge. Academic tenure is being attacked, and often attenuated. There is an unfamiliar emphasis on efficiency and good management. But the similarity between Britain and Australia is not close. The federal Australian government's plans are much more radical than any the British government has put forward.

At the heart of the changes now under way in Australia is the government's plan to create a unified system of higher education. The driving force is partly that of equity between individuals and partly that of increasing the proportion of young Australians finding their way into higher education. The July 1988 policy statement from which the events of the past year have followed says that, "in the past, the benefits of higher education have been shared disproportionately by the more privileged members of our community . . . [they must] be shared more widely and more equitably in the future". The same document argued for an increase in the annual output of graduates from 88,000 then to 125,000 by the end of the century, evoking in the process familiar disputes about the feasibility of recruiting sufficiently able students and an Australian version of the complaint that "more means worse".

Although academics may be generally sympathetic to the objectives, many of them have a sense of being railroaded into the future by the sheer speed with which their institutions are being transformed. A few years ago, there were three kinds of institutions providing some components of higher education — traditional universities with international reputations, technical and vocational colleges, also often of some distinction, and a network of institutions called technical and further education, called collectively the TAFE sector.

The coexistence of the first two sectors used to be referred to, as in Britain, as the binary system. It is now more than two years since Dawkins announced that it would cease to exist. The essence of the unified system of higher education is that all these institutions will be degree-awarding institutions, dealt with on an equal footing by the federal government, which will be the chief source of financial sup-

port for the costs of operation and capital development. But it is also planned that the TAFE sector should contribute towards the growth of higher education by providing for a proportion of students the first two years of the standard four-year university course — for which purpose, there are intended to be arrangements by which credit for courses followed shall be transferable from one institution to another.

The recipe is simple. All institutions in the higher education sector will be financed for teaching purposes on the basis of what is called their "respective educational profile rather than by institutional title" and that research support will be awarded competitively throughout the higher education sector "according to institutional performance". Although the objectives are essentially those now being pursued in Britain, the Australian plan is the more radical to the extent that the binary system has been ended by decree (for the time being, at least, there is no talk of that in Britain) and by the telescoped timetable for transition to a system in which teaching and research will separately make claims on public resources.

Amalgamation

Three particular circumstances seem to have occasioned most discontent among those academics who dissent, of which the chief is the pattern of amalgamation being forced on the higher education system. The Australian government has made no secret of its intentions. Although the number of students in higher education is to be increased, the number of distinct institutions must shrink. The rationale for this decision is simply the government's view that institutions with fewer than 2,000 students are too small to be administratively efficient, that those with fewer than 5,000 students are too small to offer a full range of courses to students and that those with fewer than 8,000 students are probably too small to win distinctive reputations in research.

But how can it be arranged that institutions with different objectives and traditions will joyfully pool their resources, people as well as property, in a merger? Canberra found a simple answer. All universities and colleges were required, in 1988, to apply for membership of the unified national system of higher education, while state governments were invited to produce plans suggesting which of their own institutions should be merged. The government was then well placed to attach conditions to membership of the unified system of higher education, which often included the requirement that particular institutions should merge with others, often those recommended for merger by the state governments.

The process of amalgamation is now

well under way. The University of Sydney, for example, will begin the new academic year as an amalgam with the Sydney Institute of Education, the Institute of Nursing Studies and the New South Wales State Conservatorium of Music, with other institutions likely soon to be brought beneath the umbrella. Both the University of Melbourne and the Melbourne College of Technology are similarly hubs for newly merged institutions. Only in Queensland, in this respect as in others a law unto itself, does the merger process hang fire.

As in all academic mergers, academic snobbery colours opinions, but there are also objective causes for concern. The university's ethos of research will be diluted and the competition for united research funds will be increased. At Sydney, it seems to be generally acknowledged that the smaller partners in the proposed amalgam are distinguished of their kind, but there is genuine concern that a faculty used to worrying about familiar academic problems may be less than adequate at worrying about the problems of a music conservatory, for example.

Some academics even try to put themselves in the shoes of the smaller partners in these mergers, asking whether the promised gains in efficiency will materialize simply by designating a small vocational school part of a much larger institution, when the management of the smaller enterprise may be well adapted to its immediate needs. Mergers that appear nuisances to large institutions may seem to entail a recipe for bureaucratic interference in well-ordered affairs by the smaller partners.

With all that said, there is a certain hysteresis in the merger process. It is more difficult to arrange a marriage, even with the help of a shot-gun, than to dissolve it. Meanwhile, it is curious that there is relatively little resentment of the arrangement that one per cent of the higher education allocations to institutions is being put into what is called a National Priority Budget, mostly being used to meet the costs of amalgamations.

Even if there is a change of government later in the year, and even if the new government were to take the view that the unified system of higher education is a mistaken goal, it is unthinkable that there would be an immediate return to the institutional *status quo*.

Bureaucracy

That principle probably also applies to another conspicuous thread in the universities' complaint against the government — that it has acted haughtily in pushing through its plans. The government denies the charge, pointing to the period of consultation allowed between the first publication of its plans for higher education (in December 1987) and the final determina-

tion of its policy (in July the following year).

But the process of discussion and consultation, all too brief, appears also to have been ritual in character, with university vice-chancellors driven to public protests by their sense, right or wrong, that other channels were deaf. That uncomfortable tradition persists, raising the forbidding prospect that even when universities have learned to live with the new arrangements, they will have acquired a habit of talking back in public that will serve nobody's long-term interests.

Part of the trouble is that Australian governments have a long tradition of listening most intently to the advice they want to hear. Early in its term of office, the Hawke government created a Commission on Tertiary Education with objectives very similar to those adopted for the Unified National System, but then abolished it four years ago when it showed signs of departing from declared policy. Have the advisory committees of the National Board for Employment, Education and Training (called NBEET, pronounced "en-beat") learned from that and other examples to tune the advice they offer to educated guesses of what would be welcome?

Meanwhile, there seems to be a general fear among academics that the creation of the unified system has provided federal civil servants with unprecedented opportunities for invigilation from the centre. The principles on which funds will in future be provided are that the costs of teaching home (but not overseas) students will be met by means of a formula itself related to the numbers of students following courses of different kinds (and inherent costs). By the same test, it will be decided centrally whether extra capital resources are required, and where they should be invested.

The difficulty is that the requirement that an institution's budget should be determined by its performance in teaching students gives the government a licence to collect the most detailed information. One vice-chancellor asks suspiciously what sinister use the people in Canberra plan to make of information now accumulating about the particular courses followed by individual named students. Another points to administrative burden on his university of answering all Canberra's questions. There are counterparts in Britain who would sympathize.

It goes without saying that not all academics or vice-chancellors are in this camp. Quite apart from approving of the federal government's objectives, there are many who also see the benefits for themselves. The University of Wollongong, for example, 60 kilometres south of Sydney, was established during an earlier period of expansion and came into being as recently as 1975. Now it has nearly

8,000 students, of whom more than 2,000 are external students or part-timers. Even so, the spanking new science building seems underoccupied.

With ambitions to form more durable links with industry than is the general rule, as well as to take more than its due share in Australia's exploitation of the market in higher education for students from overseas (chiefly from Asia), Wollongong has everything to gain from the federal government's policy on higher education: more students, more buildings and what it calls a Business and Technology Complex, a scheme for providing special training in fields as different as computer science and English as a Foreign Language to which it has also been able to attract an industrial research and development centre for microwave technology. The teaching courses provide extra income, the technology centre is a means of attracting to the site able people who pay some rent.

Claw-back

But neither the issues of amalgamation nor the indignities that follow from the doctrine of accountability appear to irritate academics as much as what is called the "claw-back" — the arrangement under which the collective higher education budget is reduced so as to stuff the coffers, as some academics see it, of the Australian Research Council (ARC). It is a curious tale, with distinctively Australian overtones.

The principle is that the allocation of research support in higher education should be competitive, one of the two pillars of the new policy on higher education. The government is ready to acknowledge that research in universities is an important part of Australia's research effort, but takes the view that neither academic researchers nor their institutions are the best judges of how funds should be spent.

Thus it is that ARC emerged (in 1988) to succeed what was previously the Australian Research Grants Committee, with a budget that grew beyond A\$20 million a year only in 1983. In the old days, the committee was a deliciously informal organization. Research grants were small, and usually awarded after personal interviews at universities by a subcommittee on a peripatration around Australia. By the mid-1980s (but, on some opinions, much earlier) it had become plain that the committee's funds were entirely inadequate to meet national needs. Too little money was spread too widely adequately to sustain all but the smallest projects.

ARC is a larger and more ambitious animal. Last year, it had a budget of its own of A\$75.5 million, but in addition its resources were increased by A\$26.6 million by transfer from the higher education budget. The plan for the years ahead is that the total budget this year will

amount to more than A\$150 million (A\$34 million of that taken from higher education) with an extra A\$25 million from the government in Canberra, and that the budget will further increase to A\$208 million by 1994 (when the claw-back will have fallen to 20 per cent of the total).

The anguish engendered by the claw-back in higher education seems poorly matched to the magnitude of the sums involved, which are of the order of one per cent of what the federal government spends on higher education. One complaint is that the higher education institutions will be rendered incapable of providing the basic facilities necessary to enable academics to compete successfully for research grants (but ARC plans to pay overhead costs at a rate of 35 per cent of the value of grants awarded). Another, perhaps more serious, is that the coincidence of the general expansion now decreed and a chronic shortage of resources will create circumstances comparable with those in Britain in which would-be academic researchers are anchored too much in front of student classes.

The more serious cause of discontent is the simple circumstance that the academic community in Australia has not yet learned to trust ARC. Part of the trouble is personal. Asked whether it is true that he is the most unpopular man in Australian academic life after Dawkins, Aitkin (startled by the question) says that the charge may have been appropriate some months ago, but is no longer. With some justice, he also protests that natural scientists do not take kindly to the notion that the chairman of ARC should be a social scientist.

But Aitkin is also an impatient figure. In a now celebrated speech in Canberra in 1987, he reminded an Australian audience that Australia, possibly the richest country in the world at the turn of the century, and certainly more prosperous than the United States and the countries of Western Europe, has allowed its pre-eminence to decline through complacency and the neglect of education. "If we needed a skill, we imported it." The consequence is that Australia's position in the world's economic pecking order had declined and would continue to do so.

Aitkin's message was then, and still is, that Australian society has neglected education, that the Australian government has followed suit and that higher educational institutions, but especially universities, have been over-concerned with academic excellence in scholarship and research. In passing, he proclaimed the virtues of a package of reforms which in retrospect is indistinguishable from Dawkinsization.

The first two years of the ARC have predictably been turbulent. One of the council's ambitions is that there should be objective means of estimating perform-

ance in research, but a long wrangle over the virtues of the *Science Citation Index* appears to have petered out, at least for the time being. Another is Aitkin's own ambition that as the budget of ARC doubles in the next five years, the number of grants awarded should not increase, but their average value should multiply by two.

Because the grants available for important projects are already smaller than they would be elsewhere, the goal seems outwardly sensible. ARC's pattern of spending is, in reality, a sensible mixture of long-term and short-term support, with a sensible understanding that able researchers may be supported for several years on long-term programme grants, free from the hassle of repeated reapplication. ARC has also been able to support some relatively large projects, having provided the largest part of the A\$2.5 million that will be required to complete the University of Sydney's large optical stellar interferometer, due to be commissioned in July or thereabouts this year.

But given the general feeling among academics that research grants are already too few to satisfy the needs of all potentially able researchers at Australian universities, and the inevitable increased demand that will be stimulated by the expansion now set in train, the anguish to be heard on every side is understandable.

The distinctively Australian character of this argument about the justice of the claw-back and the operation of ARC shows itself in several ways. For one thing, it is intensely personal: in a country still small enough, the great distances notwithstanding, for everybody of importance to know everybody else with the same pretensions, it seems natural that institutions and even committees should be invested with the personality of those in charge of them. Second, where committees, councils and even commissions can be dispensed with (or at least ignored) as easily as in Canberra, it is natural that their chairmen should be regarded as the willing tools of their ministers.

Industry

The hidden agenda in Aitkin's ARC may in any case be very much to the government's liking. If there are potentially able researchers unable to find a footing in research for lack of a grant, may they not as a consequence be driven into the arms of industry? From the beginning of the Hawke government and in the earlier proselytizing of Mr Barry Jones, it has been a constant refrain of the administration that one of Australia's troubles is the neglect by industry of the opportunities provided by research and development.

A judicious blend of exhortation and tax incentives appears to have gone some way towards redressing that balance. The latest estimate (for 1986-87) is that



Will Australia want to keep up with Barry Jones? The next election will tell.

industry and commerce then spent close to A\$1,100 million on research and development, more than twice as much as five years earlier. But the reality of some of the recent increase is open to question, given that Australia's unique 150 per cent tax credit for research and development spending (which cost the federal government A\$150 million in 1986-87) appears to have stimulated some reclassification of expenditure.

Even so, there is no prospect that the tax incentive scheme will be withdrawn. Mr Jones, a left-wing member of a left-wing government, makes no secret of the his determination that Australia should be cajoled as well as bullied into the modern world. If that requires that there should be hand-outs for industry, so be it seems to be the recipe.

Plainly, there is still a long way to go. Although industrial spending on research has increased, it amounts to less than 40 per cent of Australian spending on research and development, which is itself still less than one per cent of gross national product, only a third of the comparable ratio in Japan, West Germany and the United States. In the circumstances, it is no wonder that the managers of Australia's research should be as anxious as they are for some tangible sign that even this relatively small investment has begun to yield its expected reward.

There is a sense in which that anticipation is more widely shared. One of the most remarkable (but subjectively observed) changes in the past few years has been the emergence of a kind of research patriotism in Australia. The country has notoriously been reconciled for years to the regular loss of able people by emigration; now it is as if those who have stayed, or who have gone abroad and then returned, feel it necessary to remind themselves of the reasons why. But certainly

the Hawke government and its ministers have won powerful support for their policy of seeking to change Australia by research. It is a striking phenomenon, marked enough to survive an election with any outcome.

In the old days, this optimism used to be the sole if somewhat complacent prerogative of CSIRO, which comported itself as if its unique responsibility was to save Australia from natural and unnatural hazards, from geographical isolation and even from benign neglect. CSIRO is still the chief source of support for some of Australia's important basic research, in astronomy and atmospheric science, for example, but is being steadily edged towards strategic research of a kind that will support manufacturing industry, extant and yet to be created.

CSIRO's budget has all the while declined, to the point at which direct support from the government is no more than A\$300 million a year. (By contrast, the government claims that indirect support for university research through the recurrent budget amounted to A\$490 million in 1988, before the claw-back, but that estimate of the overhead cost of academic research is no better than the guesses made elsewhere.) The organization earns a further A\$100 million by contract work from government departments and industry as well as from its own investment in past research. The ethos is still as distinctive as that of a regimental club, but the old complacency has been lost. Maybe that has also been part of the Hawke government's agenda.

How well the calculation of the future has been made is anybody's guess. On the face of things, it seems rash to antagonize as powerful a constituency as that represented by the universities for the sake of a reorganization of higher education whose objectives are generally applauded, but whose speed and manner might have been designed to cause annoyance. Yet the government may safely, while its ambitions are unrealized, be relying on the indifference of Australia to higher education to ensure that the constituency does not count for much.

Whatever the outcome of the election, one winner will be Mr Barry Jones. Without previous experience of ministerial office, he was quickly found wanting in the skills required to harness his enthusiasm to the art of persuasion, which is a politician's skill. With the passage of time, he has won friends in higher education and research even among former enemies, while his early pleadings for a great expansion of higher education and for more research by industry seem well on the way to being fulfilled. Whether his dream that Australia could become an equal partner with Japan on the Pacific rim will ever come true is another matter, but for another decade. □

An excess of perfection

The good news is that the three-degree cosmic microwave background has the exact spectrum of black-body radiation, and is spatially featureless. The bad news is the same.

Crystal City, Virginia

EVERY now and then, data from balloon- and rocket-borne experiments have suggested that the spectrum of the three-degree background, the diluted remains of the Big Bang itself, departs a little from Planck's simple formula for radiation from a body of constant temperature. Such an indication two years ago, from the Berkeley-Nagoya collaboration, set off a spate of speculations: perhaps the reported excess of photons on the infrared side of the 2.7-K peak was the remnant of some fierce process of early cosmic history, connected with the formation of galaxies; perhaps it even signalled some fundamental flaw in Big-Bang cosmology.

The most remarkable finding delivered to the annual meeting of the American Astronomical Society last week was the revelation that COBE, the Cosmic Background Explorer satellite, has laid to rest, apparently forever, all such speculations. Less than two months into its year-long mission, COBE has given to project scientist John Mather of the NASA/Goddard Space Flight Center data that allowed him to draw a graph in which about twenty measurements of the absolute intensity of the microwave background at twenty different frequencies fall precisely on a Planck curve for black-body radiation at a temperature of 2.735 ± 0.06 K. These measurements, whose 1 per cent uncertainty will be reduced by at least an order of magnitude by the end of the year, leave the Berkeley-Nagoya excess high and dry.

That was the good news. The demise of the Berkeley-Nagoya excess was generally welcomed because even remotely plausible explanations for it had been hard to come by. Cosmologists were obliged to suppose, in one example, that there might have been an early pregalactic generation of supermassive stars, which had burned brightly but briefly before exploding as gigantic supernovae. The radiation from these stars was then to be thermalized by dust grains formed from material ejected by the supernovae. The net result of this intricate scheme was to have been an excess of radiation sitting just next to the peak of the microwave background.

Few will be sorry to say goodbye to such convoluted theories. Nevertheless, the thought that the microwave background should be perfectly thermal is not an entirely happy one. Galaxy formation, however it happens, cannot be done secretly. Galaxies may arise from the growth

of primordial density fluctuations, by the aggregation of material around loops of cosmic string, or through the triggering action of shock waves from giant supernovae, but no matter what the route, a more or less uniform distribution of material must gather together in gravitationally bound clumps, and this can happen only if gravitational potential energy is somehow disposed of.

Inevitably, as it collapses into discrete objects, gas will heat up and begin to shine; this energy redshifted to lower frequencies, must still lurk somewhere in the infrared or radio region of the cosmic spectrum. If COBE continues to see an apparently pristine spectrum of cosmic radiation, unmodified by any subsequent

energy releases, cosmologists will be forced to make their galaxies in an increasingly furtive way.

The difficulty is exacerbated by a second early result from COBE. George Smoot, of the University of California at Berkeley, revealed that another of the detectors on the satellite had so far failed to find any irregularities in the spatial distribution of the cosmic background radiation. This adds to a long litany of negative results from searches for fluctuations in the intensity of the background from one point in the sky to another. A map of these intensity fluctuations is in effect a map of the density of the Universe at an age of about 300,000 years, when the photons observed now by COBE last interacted with matter.

But in a completely uniform Universe, galaxies could never appear. Whether galaxies themselves or massive stars form first, or even if cosmic strings pull matter around, some fluctuations in density must have been present. Because there are galaxies now, in other words, there must also be non-uniformities in the microwave background — at some level and on some angular scale.

So far, nobody has found any irregularities on any scale, and COBE's preliminary data reduce the upper limit on fluctuations at an angular scale of 7° by about a factor of two. Another factor of ten improvement should come when COBE has mapped the sky in full.

Theoretical cosmologists have been enormously ingenious, of course, in modifying their ideas of galaxy formation to stay beneath the upper limits given to them by their observational colleagues. But the limits continue to descend, and the finetuning of the theories seems to be stretched almost to breaking point. And even in current theories, galaxy formation tends to proceed from initial conditions found by assertion through the medium of so many hypothetical types of dark matter that many find the whole business unappealing.

If, by the Big Bang, one means the notion of a universe expanding smoothly from a hot initial singularity, then COBE's first results are a glorious confirmation. But if one also includes twenty years' worth of sophisticated work on galaxy formation as an integral part of the Big-Bang model, then cosmologists could be in for an upsetting decade.

David Lindley

SN1987A levels off

ONE year after it exploded, supernova 1987A emitted its first gamma rays. One year later again, the notorious half-millisecond pulsar appeared and disappeared. Now, with its third anniversary coming up next month, SN1987A has revealed itself a little more. In IAU Circular 4933, astronomers from the European Southern Observatory say that the supernova's total brightness, which had been falling exponentially, has at last levelled out, indicating the presence of an internal energy source giving out about 10^{38} erg s⁻¹.

As the exponential light curve, powered by ^{56}Co synthesized in the explosion, dwindled, astronomers had been anxiously waiting for heat provided by the accretion of material onto the remnant neutron star to make itself known. The latest observations, from P. Bouchet, I.J. Danziger and L.B. Lucy, show that the infrared magnitude of SN1987A has remained essentially constant since late last year.

But this is not a confirmation that the transient submillisecond pulsar was really there. According to Stan Woosley of Santa Cruz, the estimated magnitude is suggestive of a non-pulsing neutron star radiating at the Eddington limit, the maximum it can generate without blowing the accreting material away. And even if a pulsar were now found, it would not necessarily confirm the reality of the submillisecond pulsar, whose energy output and spin-down rate were so poorly determined that it is impossible to guess what it might look like a year later.

D.L.

Mendel — now down to the molecular level

J.R.S. Fincham

UNIVERSITY teachers of genetics usually think it necessary to impart a sound knowledge of Mendel's laws, but sometimes fear that spending time on these foundations of the subject will tax the patience of their pupils. First-year students tend to think (even if mistakenly) that they have already had enough mendelism at school, and they are understandably eager to hurry on to the most recent advances in molecular genetics and gene cloning. A recent paper¹ by a group at the John Innes Institute, Norwich, could be a boon to teachers looking for examples to help them bridge the gap between the old and the new.

One of the seven single-factor differences upon which Gregor Mendel based his seminal study of inheritance in peas was that governing seed shape: the dominant round (*R*) versus the recessive wrinkled (*r*). It is a particularly convenient example for demonstration. Because the difference resides in the fleshy seedling leaves or cotyledons of the coming generation, the classical mendelian ratios (3:1 in an F₂ generation and 1:1 in a backcross) can already be seen in the seed-pods.

The John Innes group has now analysed the determination of the wrinkled phenotype at all levels. Like similar seed phenotypes in other plants (the wrinkled seed of sweet-corn is the most familiar example) it is thought to result from a high sugar content, which in turn leads to increased accumulation of water and greater room for shrinkage on drying. In the case of wrinkled peas, the high sugar content is due to a defect in starch synthesis, due in turn to the absence of an enzyme, starch-branching enzyme I (SBEI), that normally produces branched-chain amylopectin to supplement the straight-chain amylose. As well as the deficiency in amylopectin and high sucrose content, wrinkled seeds have peculiarities of protein and lipid content that are less well explained. It seemed obvious, however, that the lack of SBEI was at the root of the wrinkled syndrome.

Accordingly, antiserum prepared in rabbits against the purified enzyme was used to screen a pea embryo complementary DNA library cloned in an *Escherichia coli* expression vector. The SBEI-

encoding cDNA was indeed recovered. Using the longest cDNA clone as probe on northern blots of messenger RNA and Southern blots of genomic restriction endonuclease digests, the authors were able to show that, in comparison with *RR* plants, the SBEI gene transcript in *rr* plants is unusually large as well as much reduced in quantity, and that the corresponding genomic DNA sequence has an insert of 0.8 kilobases (kb). This size difference was shown to segregate from crosses with the *r* locus, as if the *r* mutation

of a sequence that is a member of a multi-copy sequence family.

Sequencing of the relevant portions of the 3.3-kb and 4.1-kb clones revealed that the 0.8-kb insert in the latter had inverted terminal repeats of 12 base pairs and was flanked by an eight-base-pair tandem duplication evidently generated from a single-copy sequence at the site of insertion. Inverted terminal repeats and host-site tandem duplications are hallmarks of the transposable DNA elements that have been characterized from several plant species, especially *Zea mays* and *Antirrhinum majus*². Furthermore, the inverted terminal repeats of the pea 0.8-kb sequence matched, in seven to nine out of 12 positions, those of several movable elements from other plants — the maize Ac/Ds family, antirrhinum Tam3 and parsley Tpc1.

The size of the insertion in the *r* allele seems rather small for an element encoding its own excision/transposition apparatus. In the maize Ac/Ds system the Ds elements are mostly deletion derivatives of Ac and are only transposable if complemented in *trans* by a full Ac element. One may conjecture that the 0.8-kb sequence is analogous to Ds and would be able to transpose out of the *r* locus if any of the multiple copies detected by the 4.1-kb probe were the equivalent of a fully functional Ac element. In fact, an excision might well not restore gene function; the insertion is said to be located within an exon of the SBEI gene and would most probably leave behind some or all of the eight-base-pair tandem repeat, with the probable effect of a frameshift mutation. But it has evidently not excised in this way in the long history of the present *rr* stocks, and the presumption must be that the mutation is stable. It may well be that the 0.8-kb insertion is a representative of an originally mobile family of repeats that are no longer mobile in the present pea strains because of decay by mutation or deletion, or possibly by methylation. But this remains to be seen; it would be interesting to see a comparison of the sequences of several of the present members of the family.

The findings of the John Innes group add further to the plausibility of two propositions: first, that transposable elements of the kind first identified by Barbara McClintock in maize are widely distributed in plants and not just rare exceptions; second, that the activities of such elements are a major source of spontaneous mutation in many species. Had



Gregor Mendel, standing second from right, with members of the Augustinian Monastery in Old Brno from 1861 – 64. Taken from *Iconographia Mendeliana* (courtesy of R. Olby).

were due to an insertion of an extraneous sequence into the wild-type *R* gene.

The nature of the insertion was investigated by cloning *EcoRI* fragments, hybridizing to a subclone of the cDNA, from both *RR* and *rr* plants. A single fragment was cloned from each genotype, of size 3.3 kb and 4.1 kb from *RR* and *rr* plants respectively. When these clones were used in turn as probes on genomic *EcoRI* digests, the 3.3-kb sequence hybridized to single 3.3-kb and 4.1-kb fragments from *RR* and *rr* respectively, but the 4.1-kb fragment recognized some 30 or more fragments of similar sizes in each genome, except for a 4.1-kb fragment again seen in *rr* but not in *RR* DNA. This showed that the *r* mutation was probably due to the transposition into the *R* locus

it already been available, the newly discovered element could have been used as a 'tag' to aid the cloning of the *r* gene. Perhaps some other important pea mutants are tagged in the same way.

Is this wrinkled mutant, which so pleasingly brings together the old and the new in genetics, really the same one as studied by Mendel? The authors believe so, because the present *r* mutation is widely distributed among European cultivars and there is no evidence that any other

ATMOSPHERIC SCIENCES

mutant of similar phenotype was available in Europe in Mendel's day. But even if there is a shadow of a doubt, it seems unlikely that teachers of genetics will allow that to spoil a good story □

J.R.S. Fincham is in the Department of Genetics, University of Cambridge, Downing Street, Cambridge CB2 3EH, UK.

1. Bhattacharyya, M.K., Smith, A.M., Ellis, T.H.N., Hedley, C. & Martin, C. *Cell* **60**, 115–122 (1990).
2. Berg, D.E. & Howe, M.M. (eds) *Mobile DNA* (Am. Soc. Microbiol., Washington, DC, 1989).

Chemistry with a silver lining

Stephen E. Schwartz

CLOUDS occupy only about a tenth of the volume of the troposphere, and of that volume, only about a millionth is occupied by condensed water. So it may seem surprising that reactions occurring in the condensed phase in clouds can substantially alter the chemistry of the atmosphere. Nonetheless, this seems to be the case. For some years it has been recognized that oxidation of SO₂ to sulphuric acid, an important component of the overall acid deposition process, occurs rapidly in liquid-water clouds when the highly soluble hydrogen peroxide, a strong oxidant, is present^{1–3}. More recently it has been argued convincingly that a reaction on ice particles during the polar stratospheric night converts relatively inert ClONO₂ and HCl into photolabile species ultimately responsible for ozone depletion^{4,5}. Now Lelieveld and Crutzen present, on page 227 of this issue⁶, model calculations that indicate that aqueous-phase reactions in clouds substantially affect the budget of ozone in the troposphere. Ozone's central role in tropospheric chemistry makes this a result of great significance.

The gas-phase tropospheric chemistry of ozone, and that of species responsible for its production and destruction — nitrogen oxides, hydrocarbons and CO, and free radicals, principally OH and HO₂ — has been thought to be fairly well understood. Net ozone production occurs by free-radical-initiated oxidation of NO such as indicated in cycle I in the table. Net destruction also occurs by free-radical processes, cycle II. Ozone is a precursor to free-radical formation, cycle III, giving rise to the adage that it takes ozone to make ozone; it also takes ozone to destroy ozone. Free radicals are formed also by photolysis of aldehydes, cycle IV. Elucidation of this chemistry, together with knowledge of the spatial dependence of sources of nitrogen oxides, hydrocarbons and CO, has enabled atmospheric chemists to construct models that seem to describe more or less successfully the chemistry of ozone and

related compounds in the global troposphere^{7,8}.

Examination of the influence of in-cloud reactions on tropospheric ozone chemistry requires both a framework for coupling chemistry in the two phases present and knowledge of the solubilities, interfacial mass-transport kinetics and aqueous-phase kinetics of the pertinent species. Lelieveld and Crutzen have drawn upon recent advances in each of these areas, gained largely in pursuit of improved descriptions of acid-rain chemistry. They incorporate this process-level understanding into a cloud-climate

model resolved latitudinally and vertically to obtain an initial assessment of the influence of cloud chemistry on the budget of ozone in the global troposphere. Aqueous-phase reactions seem both to inhibit the production of ozone, by decreasing free-radical production from formaldehyde photolysis and by catalysing loss of free-radicals (cycle V) and nitrogen oxides, and to enhance ozone destruction (cycle VI). Relative to calculations that neglect this cloud chemistry, ozone production is decreased by about 40 per cent and, depending on conditions and assumptions, the effective rate coefficient describing ozone destruction is increased by 30 to 270 per cent — no minor adjustment. The inclusion of cloud chemistry also substantially decreases calculated concentrations of formaldehyde, nitrogen oxides and OH and HO₂ free radicals. By examining the implications of cloud chemistry on the global budgets of these compounds, Lelieveld and Crutzen have made an important advance over earlier studies (for example, refs 9–11) that focused on the chemistry itself.

Given the small amount of liquid water in the troposphere, why should aqueous-phase reactions be as important as is suggested by Lelieveld and Crutzen's work? A first clue is the high solubility of a few key species that results in an

Some important reaction cycles governing tropospheric ozone chemistry

Gas phase	Gas phase continued
Cycle I: ozone production	Cycle IV: free-radical production from formaldehyde
$\text{HO}_2 + \text{NO} \rightarrow \text{OH} + \text{NO}_2$	$\text{H}_2\text{CO} \xrightarrow{h\nu} 2\text{H} + \text{CO}$
$\text{NO}_2 \xrightarrow{h\nu} \text{NO} + \text{O}$	$2(\text{H} + \text{O}_2 \rightarrow \text{HO}_2)$
$\text{O} + \text{O}_2 \rightarrow \text{O}_3$	Net: $\text{H}_2\text{CO} + 2\text{O}_2 \rightarrow 2\text{HO}_2 + \text{CO}$
$\text{OH} + \text{CO} \rightarrow \text{CO}_2 + \text{H}$	Aqueous phase
$\text{H} + \text{O}_2 \rightarrow \text{HO}_2$	Cycle V: free-radical sink
Net: $\text{CO} + 2\text{O}_2 \rightarrow \text{CO}_2 + \text{O}_3$	$2\text{HO}_2 \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$
Cycle II: ozone destruction	$\text{OH} + \text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + \text{HO}_2$
$\text{O}_3 + \text{HO}_2 \rightarrow \text{OH} + 2\text{O}_2$	Net: $\text{OH} + \text{HO}_2 \rightarrow \text{H}_2\text{O} + \text{O}_2$
$\text{O}_3 + \text{OH} \rightarrow \text{O}_2 + \text{HO}_2$	Cycle VI: ozone destruction
Net: $2\text{O}_3 \rightarrow 3\text{O}_2$	$\text{HO}_2 \rightarrow \text{H}^+ + \text{O}_2^-$
Cycle III: free-radical production from ozone	$\text{O}_2^- + \text{O}_3 + \text{H}_2\text{O} \rightarrow 2\text{O}_2 + \text{OH} + \text{OH}^-$
$\text{O}_3 \xrightarrow{h\nu} \text{O}(^1\text{D}) + \text{O}_2$	$\text{OH} + \text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + \text{HO}_2$
$\text{O}(^1\text{D}) + \text{H}_2\text{O} \rightarrow 2\text{OH}$	$\text{H}^+ + \text{OH}^- \rightarrow \text{H}_2\text{O}$
Net: $\text{O}_3 + \text{H}_2\text{O} \rightarrow 2\text{OH} + \text{O}_2$	Net: $\text{O}_3 + \text{H}_2\text{O}_2 \rightarrow 2\text{O}_2 + \text{H}_2\text{O}$

equilibrium partition into the aqueous phase which greatly exceeds the volume ratio of the two phases. This applies especially to a species such as the hydroperoxyl radical HO_2 , which in aqueous solution undergoes weak acid dissociation to form superoxide ion, O_2^- . This dissolution separates soluble and insoluble species that would otherwise react in the gas phase, such as HO_2 and NO . More importantly, it concentrates reagents in solution, enhancing second-order reactions. This concentration, together with a decreased activation energy for the reaction, results in high fluxes through certain aqueous-phase reactions despite the low liquid-water volume fraction. Clouds also reactively dissolve N_2O_5 , thereby irreversibly converting reactive nitrogen oxides into unreactive nitric acid. All of these processes work to decrease ozone concentrations.

Lelieveld and Crutzen's paper will hardly be the last word on this important subject. Future work will have to examine the influence of aqueous-phase composition, including effects of pH, which affects HO_2 solubility, and of transition metals, which can catalyse HO_2 disproportionation. More troublesome are the implications of this work for the budgets of ozone and nitrogen oxides. If we are to believe that aqueous-phase fluxes are as great as Lelieveld and Crutzen suggest, then other processes will

have to be identified to account for the observed concentrations of these species. In fact, the budget imbalances are even worse than Lelieveld and Crutzen indicate. In their model, ozone concentration was maintained at values representative of the cloud-free atmosphere rather than being allowed to decrease, as that would have resulted in ozone concentrations much lower than observed. Similarly calculated nitrogen oxide concentrations were much too low if clouds were allowed to scavenge N_2O_5 . These discrepancies will have to be resolved before confidence can be restored in our understanding of the tropospheric budgets of these substances. □

Stephen E. Schwartz is in the Environmental Chemistry Division, Brookhaven National Laboratory, Upton, New York 11973, USA.

1. Penkett, S.A., Jones, B.M.R., Brice, K.A. & Eggleton, A.E. *J. Atmos. Environ.* **13**, 123–137 (1979).
2. Schwartz, S.E. in *SO₂, NO and NO₂ Oxidation Mechanisms: Atmospheric Considerations*, (ed. Calvert, J.G.) 173–208 (Butterworth, Boston, 1984).
3. Calvert, J.G. *et al. Nature* **317**, 27–35 (1985).
4. Molina, M.J., Tso, T.-L., Molina, L.T. & Wang, F.C.-Y. *Science* **238**, 1253–1257 (1987).
5. Tolbert, M.A., Rossi, M.J., Malhotra, R. & Golden, D.M. *Science* **238**, 1258–1260 (1987).
6. Lelieveld, J. & Crutzen, P.J. *Nature* **343**, 227–233 (1990).
7. Liu, S.C. *et al. J. Geophys. Res.* **92**, 4191–4207 (1987).
8. Isaksen, I.S.A. & Hov, Ø. *Tellus* **39B**, 271–285 (1987).
9. Chameides, W.L. *J. Geophys. Res.* **89**, 4739 (1984).
10. Graedel, T.E., Mandich, M.L. & Weschler, C.J. *J. Geophys. Res.* **91**, 5205–5221 (1986).
11. Jacob, D.J. *J. Geophys. Res.* **91**, 9807–9826 (1986).

HIGH-TEMPERATURE SUPERCONDUCTORS

Critical currents pinned down

David Larbalestier

ONE of the main impediments to the application of the new high-temperature (high- T_c) superconductors is the disappointingly low current they can carry. Above a certain critical current, the desirable zero resistance of the superconducting state is lost. The study of this limit in the new materials was the subject of a recent meeting*, at which many encouraging developments were reported. These included the passage of much improved current densities (more than 10^4 A cm^{-2}) in silver-sheathed polycrystalline tapes of bismuth-based compounds at a temperature of 4.2 K and in strong magnetic fields (10–25 tesla), and in melt-processed yttrium-based samples at 77 K and 1 T.

The critical current density is considerably more complex to describe in high- T_c superconductors than in their low-temperature counterparts. At the root of the problem are two issues. The first is the granular nature of the materials, which has been evident since the first studies on the new oxide superconductors. This was first thought to be an artefact of the early,

poor preparation methods; however, it was soon shown that apparently clean crystallographic grain boundaries were weak links in the superconducting network¹. But other groups have shown that subdivision occurs on a finer scale than the crystallographic grain².

The second issue is related to the magnetic properties of the materials — which are so-called type II superconductors. In a type II superconductor, full field exclusion (except in a thin surface layer) occurs only up to a weak field H_{c1} (typically 0.05 T). Beyond H_{c1} the material enters a two-phase state in which the magnetic field exists as quantized vortices (fluxoids). The cores of the vortices can be considered normal. The density of the vortices increases with increasing field; at the upper critical field H_{c2} the whole sample becomes normal as the cores overlap. Pinning the vortices to lattice defects (from which they may be displaced by magnetic forces assisted by thermal activation) is required to develop a bulk critical current density. The complex nature of the granular state means that several types of critical current can be

Terms for describing critical current density

• **Depairing.** Above the depairing critical current density J_{cd} , the current delivers sufficient momentum to break up the 'Cooper' pairs of electrons that are responsible for superconductivity. J_{cd} can be related to the critical magnetic field H_c and the magnetic penetration length λ , as $J_{cd} \approx H_c/\lambda$. For superconductors in the Meissner state ($H < H_c$) this limit applies to the surface of the sample to a depth of about λ . In the vortex state, it applies to currents circulating around the flux lines. This is the highest critical density possible in a superconductor, and at about $5 \times 10^8 \text{ A cm}^{-2}$ for yttrium-barium copper oxides (YBCO), is about 100–1,000 times that needed for applications.

• **Flux pinning.** For fields above H_{c1} , the sample becomes permeated with magnetic flux lines. If these are uniformly distributed, Maxwell's equation ($-\mu_0 J = \nabla \times B = 0$; μ_0 is the permeability of free space) shows that a macroscopic current cannot be sustained. By pinning flux lines to defects in the samples, a non-uniform distribution is ensured so that the field gradient $\nabla \times B$ is non-zero and a bulk critical current can be

defined, as described in the box, above.

High transport current densities (over 10^6 A cm^{-2}) have been measured in epitaxial thin films of high- T_c superconductors in which the copper oxide layers lie in the plane of the film, even at fields in excess of 10 T (at 4 K). For many, such experiments constitute the proof that high critical currents can be achieved in high- T_c superconductors. But the true significance of these results remains in doubt, because it is seldom clear whether the supercurrent is crossing grain boundaries (the weak links in polycrystalline samples; see box) and if so, whether those boundaries have favourable low angles of mismatch.

Bulk, polycrystalline samples, in contrast, have been disappointing. The transport critical densities at 77 K — 10^3 A cm^{-2} in zero field and below 10 A cm^{-2} in fields of more than a few millitesla — compare poorly with the equivalent values in bulk Nb-Ti at 4.2 K (10^6 A cm^{-2} in 1 T). The prevalent practice has been to report J_c for zero field, in part to circumvent the rapid deterioration with increased field. This is regrettable for many reasons: what limits J_c at low applied field is unclear; self-field effects are strong; and field gradients across the samples are large. It is much more helpful to apply large fields (1–10 T) and to conduct the experiments in liquid helium (4 K) as well as liquid nitrogen (77 K). Measurements at 4 K minimize flux creep and show whether the granular behaviour of the superconductor is temperature dependent.

There has been substantial progress

*Critical currents in high- T_c superconductors, Nuclear Research Centre, Karlsruhe, 24–25 October 1989.

passed. The limit is then determined by the strength with which flux lines are pinned. In optimized low-temperature materials, such as Nb-Ti or Nb₃Sn, it is typically 10^{-2} – 10^{-3} times J_{c0} .

• **Grain boundaries.** When measured directly as the current transported in granular superconductors, the transport critical current J_{ct} is limited by the weakly coupled interfaces that define the boundaries of the superconducting grain network. Typically this limit is 10^{-3} – 10^{-5} times the flux-pinning critical current density.

• **Magnetization.** The critical current can also be calculated from the magnetization M of the superconducting sample. In conventional materials the value derived (J_{cm}) is much the same as J_{ct} , but for granular materials it is biased by current loops inside the grains (where $M \approx J_{ct} \times a$, a being the scale radius of the loop). Because the loop size is poorly defined (it decreases with increasing field and temperature), a precise value of the intra-grain critical current cannot always be obtained. Typically the crystallographic grain size is used for a , in which case J_{cm} will be underestimated if the crystallographic grain is subdivided into smaller superconducting grains. D.L.

during recent months, however. Significantly, two of the advances are for superconductors in prototype formats for applications — for YBa₂Cu₃O_{7-x} and Bi₂Sr₂CaCu₂O₈ made in silver tubes. The work on YBCO in Ag (K. Osamura, Kyoto Univ.) shows that wires could have J_{ct} (10 T, 4.2 K) of the order of 10^3 A cm⁻² (ref. 3). K. Heine (Vacuumschmelze, Hanau) and colleagues found that BSCCO wires could have J_{ct} (10–26 T, 4.2 K) greater than 10^4 A cm⁻² (ref. 4). Neither of these results is as good as obtained for the best thin films; but what is of the highest importance is that the values are measured across many grains in clearly polycrystalline material. Some partial texture is reported in the YBCO wires, none in the BSCCO wires.

At 77 K in a magnetic field, neither wire carries a significant J_{ct} . The wires are then granular and flux creep seems to be significant in the BSCCO wires. There was extensive discussion of these two points at the meeting. It is not clear whether flux creep is an intrinsic or an extrinsic problem.

Condensed-matter physicists tend to see it as an intrinsic problem. Operation of the new materials at 77 K, relatively close to the transition temperature, clearly makes thermal depinning of fluxoids more likely than it is in conventional superconductors, which are operated in liquid helium, well below their transition temperature ($T/T_c \approx 0.5$). Complications arise because the measured activation energy for depinning in the oxide superconductor

samples varies across a wide range (0.01–10 eV).

Particularly encouraging in this context have been studies on melt-processed samples of YBCO. Samples produced by M. Murakami and colleagues (Nippon Steel/ISTEC) and by K. Salama *et al.* (Houston Univ.) seem to show that the very large grains (1 cm long in the copper oxide plane) possible by this route do permit very large transport current densities *within* the grain (up to 3.5×10^4 A cm⁻² at 77 K, 1 T), although the critical current across grain boundaries is much less (10^3 A cm⁻² at 1 T, 77 K). Some recent elegant work by H. Kupfer *et al.* (Nuclear Research Institute, Karlsruhe) on a Houston sample does show that large J_{ct} values can be obtained across grain boundaries perpendicular to the copper oxide plane (10^4 A cm⁻² at 1 T, 77 K). But the misorientation between grains is so small that these boundaries should again be considered to be special low-angle ones with a low barrier to current flow.

The melt-processed samples produced by the Nippon Steel/ISTEC and Houston groups are notable in two other respects. First, flux creep is very much slower than for single crystals or bulk polycrystalline samples (the activation energy can be as high as 2 eV at 77 K). Second, these

samples provide the first unambiguous evidence of the avoidance of intra-grain weak links at 77 K in fields as high as 1 T. This is very important, because most of the previous studies that have explicitly looked for weak links at a level smaller than the crystallographic grain have found them. Indeed, investigations by colleagues and myself of a good single crystal show a progressively granular behaviour as temperature and field are increased.

A final encouraging result⁵ is that neutron irradiation of YBCO single crystals (R. B. van Dover, AT&T Bell Labs) can raise J_{ct} at 77 K, 0.9 T from 6×10^3 A cm⁻² to 6×10^5 A cm⁻². Thus, it seems that the raising of J_{ct} by the introduction of microstructural defects in a controlled fashion may improve the new materials as it has conventional superconductors in the past. □

David Larbalestier is at the Applied Superconductivity Center, University of Wisconsin, Madison, Wisconsin 53706, USA.

1. Dimos, D., Chaudhari, P., Mannhart, J. & LeGoues, F.K. *Phys. Rev. Lett.* **61**, 219–222 (1988).
2. Daeumling, M., Seuntjens, J.M. & Larbalestier, D.C. *Appl. Phys. Lett.* **52**, 590–591 (1988).
3. Osamura, K., Takayama, T. & Ochiai, S. *Superconductor Sci. Technol.* **2**, 107 (1989).
4. Heine, K., Tenbrink, J. & Thoenner, M. *Appl. Phys. Lett.* **55**, 2441–2443 (1989).
5. Van Dover, B. *et al. Nature* **342**, 55–57 (1989).

CONSERVATION BIOLOGY

Learning from saving species

Jared M. Diamond

JUST as one's ability to repair a car tests one's understanding of car engines, so one's ability to restore a damaged ecological community is the ultimate test of whether one understands its organization. This point was doubly illustrated at a conference arranged by New Zealand's Department of Conservation (DoC)*. Although ecological understanding is proving increasingly important for success at saving New Zealand species, conservation programmes are also yielding otherwise unattainable insights into community structure.

Because of New Zealand's isolation, area and age, its biota is richer and more distinctive than that of any other oceanic island. Endangered endemic vertebrates include kiwis, the kakapo (a flightless, nocturnal, lek-breeding parrot), tuataras (sole survivors of one of the four extant reptilian orders), and leiopalmatid frogs. The main threat besides habitat destruction comes from introduced mammalian pests — especially rats, cats, possums, weasels and ungulates — which prey on, browse on or compete with native species. Because it is now virtually impossible to eradicate such pests from the New

Zealand mainland, much conservation work has involved the transfer of endangered populations to pest-free offshore islands.

As an example of the resulting insights into community ecology, consider the eradication of Pacific rats (*Rattus exulans*) from Korapuki Island in 1986. Because nearby Stanley Island still has rats, while Middle Island has never had rats, effects of rat predation on lizard populations could be quantified both by a before-and-after comparison of Korapuki (demonstrating short-term effects of rat eradication) and by simultaneous comparison of Middle against Stanley and Korapuki-before (demonstrating long-term effects of rat introduction).

These comparisons yielded four conclusions (D. Towns, DoC). First, Pacific rats can exterminate lizard populations, as shown by Middle Island having twice as many lizard species (ten gecko and skink species on a 10-hectare island) as Korapuki or Stanley. Second, the effect of Pacific rats is selective in ways consistent with their nocturnal and ground-nesting habits: nocturnal ground-dwelling lizards are most affected. Only 8 per cent of lizard biomass was nocturnal on rat-infested Korapuki, compared to 75 per cent on

*Ecological Restoration of New Zealand Islands, Auckland, 21–24 November 1989.

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Endangered species — a male of a newly discovered species of weta from Middle Island, New Zealand. Known as the elephant weta, this species takes its name from its long tusks, used for fighting among males.

rat-free Middle. Third, the vulnerability of lizards to rats varies among habitats. After rats were removed from Korapuki, lizard densities in forest did not change (because rats had already been able to exterminate susceptible lizard species there) but they increased tenfold on stony beaches (because some individuals of susceptible species had been able to survive). Finally, because larger lizards are more vulnerable to rat predation, it was initially surprising that the shore-skink size-distribution on Korapuki Island shifted from unimodal to bimodal after the removal of rats. The explanation: there was now a higher proportion of mature female lizards, hence more production of juveniles.

Although the eradication of predators is clearly desirable when the predator threatening endangered endemic species is an introduced pest, two examples illustrate the agonizing choices that arise when the predator itself is an endangered endemic. One example (E. Kennedy, DoC) involves the Stewart Island weka, an endangered flightless bird that resembles a chicken. A population introduced from Stewart Island to nearby Codfish Island thrived and seemed ideal for safeguarding the weka's future, until it was realized that wekas were decimating populations of Cook's petrel and mottled petrel, two declining New Zealand seabirds. The controversial decision was reached to eradicate wekas on Codfish, even as wekas have been declining towards extinction on Stewart Island itself and as weka releases have continued on other nearby islands posing ecological conflicts similar to those faced on Codfish Island.

The other example (M. McIntyre, DoC) involves the recent discovery of a remarkable new species of weta (not to be confused with weka), a group of flightless, cricket-like insects of up to 70 grammes in weight. The newly discovered species, known as the elephant weta because of the tusks that males use to fight each other (see figure), is so distinctive that it may

belong to a new subfamily. It is now confined to an island of just 10 hectares, where the top predator, for which wetas are preferred food, is — alas — the tuatara, New Zealand's most important endangered reptile.

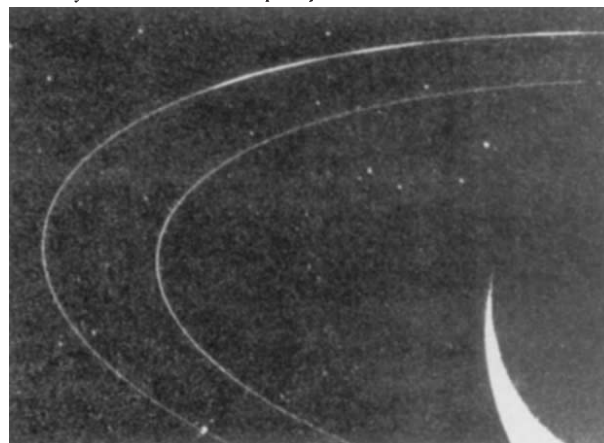
Threats to endangered species are often so urgent that one tries to save the species somewhere, anywhere. But as these two examples illustrate, the very successes of New Zealand's conservation biologists, combined with New Zealand's many endangered species and finite number of islands, mean that New Zealanders must now carefully evaluate the complex problem of interactions between endangered species before they transfer more species to islands. In particular, concentrating attention only on charismatic large vertebrates ignores or even damages populations of ugly or cryptic invertebrates of equal conservation value.

VOYAGER MISSION

More surprises in Neptune data

Clark R. Chapman

THE first detailed presentation of results since Voyager's historic encounter with Neptune last August was a session on the opening day of the recent American Geophysical Union meeting*. Although some obvious results, and instant speculations, had been offered to the world via daily press conferences during the encounter, much of Voyager's contribution will take years to develop through detailed scientific analyses of the data. Research during the three months immediately following the encounter has already led to some new perspectives.



A Voyager 2 image of Neptune's ring system showing clearly three ring arcs. (Courtesy of the Jet Propulsion Lab.)

During Voyager's encounter, Neptune was revealed as a planet whose variations in winds at different latitudes are not too dissimilar from those of the other outer planets. The only surprise at the time was the revelation that the radio rotation

Experimental eradications and transfers of species have been valuable research tools for ecologists seeking to understand community structure. But many obstacles — practical, financial, legal, political and moral — severely restrict such studies, often to areas of only a few square metres, and the resulting tiny scale may mean that the conclusions cannot be extrapolated to scales of interest. Ecological restoration in the name of conservation provides cases where it is considered virtuous to introduce or exterminate species on large islands. As the rat-lizard study reported by Towns shows, the resulting 'experimental design' may be as appropriate as if the work had been planned for purely intellectual motives. □

Jared M. Diamond is in the University of California Medical School, Los Angeles, California 90024, USA.

period, reflecting the deep interior of the planet, was shorter (at just over 16 hours) than the periods revealed by atmospheric features at practically every latitude. Virtually the entire atmosphere seemed to be "ripping to the west", according to Andrew Ingersoll (California Institute of Technology).

Since then, Ingersoll and colleagues have measured motions of cloud features within the major spots. They move in an analogous fashion to terrestrial mountain lee-wave clouds, where individual cloud features stream through the standing cloud. "And move they do!" says Ingersoll, who referred to Neptune as having a "Mach-1 atmosphere". Elements move at velocities ranging from 300 m s^{-1} towards the east to 600 m s^{-1} towards the west, with differential winds approaching the speed of sound.

Although such behaviour initially seems surprising, because it implies that there is very little dissipation of energy in the flow, Ingersoll claimed to see no problem. But it will take further research, including

numerical modelling of Neptune's atmospheric flow, to enable us to understand why Neptune is the windiest (or, at least, second windiest) planet in the Solar System.

Another changing perspective concerns the rings around Neptune and their relationship to the planet's population of small satellites. Before the encounter,

*American Geophysical Union, fall meeting, San Francisco, 4 December 1989. See also *Science* **246**, 1417–1501, 1989.

there were predictions that Voyager might find more moonlets near Neptune than exist near any other planet. One idea had been that such satellites would help to confine the then-hypothesized ring-arcs. Ring-arcs were discovered (see figure) and found to be more dense segments of a continuous ring; the physical mechanism for their confinement remains mysterious.

Rather few smaller moons were found by Voyager (a total of six), but it had been expected that more detailed analysis of the Voyager pictures would reveal quite a few more. Not so, according to Jeff Cuzzi (NASA Ames Research Center). Compared with a normal power-law size distribution expected for a population of fragmented moons, Neptune had too few 15-km-diameter satellites.

This is all the more surprising to Cuzzi, because photometric observations show that many of the rings have an unexpectedly large fraction of dust. This seems to require a continuing high rate of bombardment of the larger ring particles to replace the dust swept up by larger particles and possible moonlets. Even a hundred-fold increase in cometary flux at Neptune compared with Saturn's cannot generate the observed amount of ring-arc dust. Cuzzi believes a local source of bombarding material is necessary, presumably from a population of fragments of small moonlets. That is one reason why the apparent lack of small moonlets is so perplexing.

Neptune's largest moon, Triton, continues to astonish. When news of Triton disappeared from the front pages at the end of the encounter, it already seemed to be a fascinating world. Larry Soderblom (US Geological Survey, Flagstaff, Arizona) had speculated that there might even have been geyser-like eruptions occurring on Triton within the past century. A further three months of scrutiny of Voyager pictures begin to make Triton look more like a rival of Io insofar as activity is concerned.

Soderblom presented video sequences at the meeting, demonstrating the three-dimensional aspects of two active geyser-like plumes rising 8 km above Triton's surface, including hints of actual downstream motion of plume material in successive frames. And that was not all. There are hints of additional plumes and dark condensate clouds. Furthermore, Candy Hansen (Jet Propulsion Laboratory) has identified what may be a global distribution of plumes and related surface markings. There is evidence that the active plumes are near latitudes 50–56° south. Because Triton's south pole is tipping ever more toward the Sun this decade, there is suspicion that the plumes are triggered, possibly even driven, by solar energy.

Ingersoll (*Nature*, submitted) has been addressing another mystery about Triton's

plumes: both of the well-observed plumes trail westwards, whereas the dark surface streaks, hypothesized by Soderblom during encounter week to be due to plumes, trend towards the north-east. Ingersoll believes these observations are consistent with a general circulation model for the incredibly thin atmosphere of Triton in which the winds are westward at high altitudes (at the estimated 8 km

height of the so-called West Plume) but are northeasterly below. Although much progress in elucidating Triton's plume behaviour was reported, there are many questions remaining about the generation of this unexpected activity in one of the most frigid places in the Solar System. □

Clark R. Chapman is at the Planetary Science Institute, 2421 East 6th Street, Tucson, Arizona 85719, USA.

BIOMINERALIZATION

Iron and the origin of life

R.J.P. Williams

PART of the enduring fascination of iron minerals lies in their magnetic properties, and part in their technological applications in materials. A further interest, almost a curiosity, is the diversity of form, chemical and physical, shown by iron oxides and sulphides. In the past few years it has become apparent that the great value of iron oxides was employed in biological systems millions of years ago. Magnetite (Fe_3O_4) is known in many bacteria, goethite ($\text{FeO}(\text{OH})$) strengthens limpet teeth, and ferritin or ferrihydrite ($\text{Fe}(\text{OH})_3$ or a similar formulation) is ubiquitous as an iron store. Until recently it was thought that the biological magnetic iron minerals were found only in bacteria in relatively well aerated, aqueous parts of the Earth's surface such as fresh or sea water. But in last week's issue of *Nature* Fassbinder *et al.*¹ reported finding magnetic bacteria in relatively large numbers in soils; the authors even suggested that much of the magnetic material present in soils today, which was previously con-

sidered to be of 'inorganic' origin, arises from these soil bacteria and that although some is still intracellular much is particulate, 'fossil' material. This week, on pages 256 and 258, two other groups^{2,3} describe magnetic bacteria from the sulphide-rich layers at the bottom of stagnant water. The two papers constitute a remarkable extension to our knowledge, in reporting the discovery of several forms of iron sulphides in living organisms, including magnetic greigite (Fe_3S_4) (ref. 3) or possibly pyrrhotite (ref. 2) and pyrites (FeS_2) (ref. 3). The organisms concerned belong to the extremely primitive sulphur bacteria and live in anaerobic conditions.

Of immediate interest is the intriguing problem of how these biological systems have gained precise control over the type of mineral deposited⁴. It is no easy matter to see how it is possible to crystallize one, let alone two, chosen forms of iron oxide or iron sulphide in a cell, even in separate vesicles. We must suppose that there are organic gene products to manage

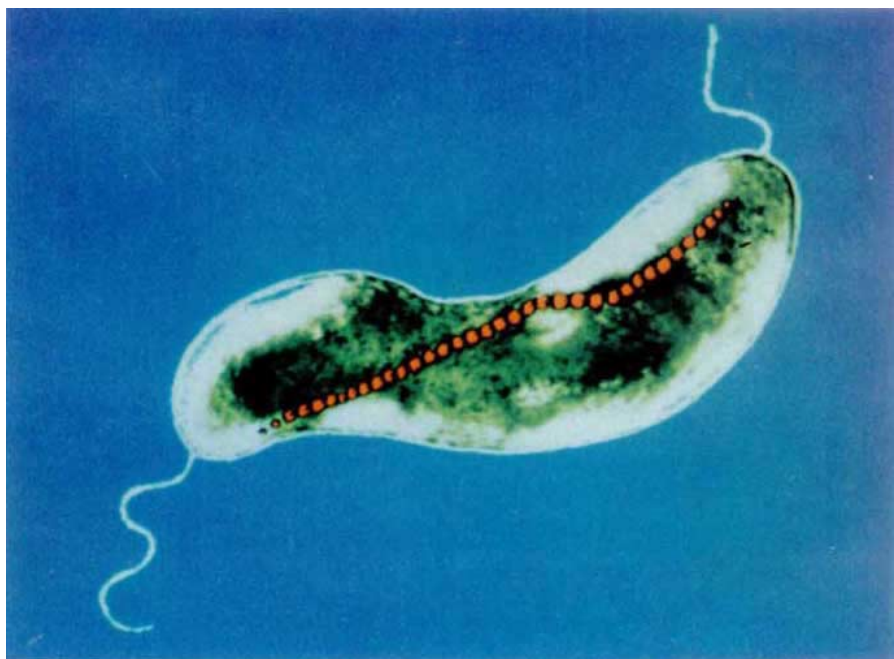


FIG. 1 Magnetic personality — false-colour electron micrograph of a magnetic bacterium from soil. The single chain of 36 magnetosomes is picked out in orange. (See ref. 1.)

H. Vail

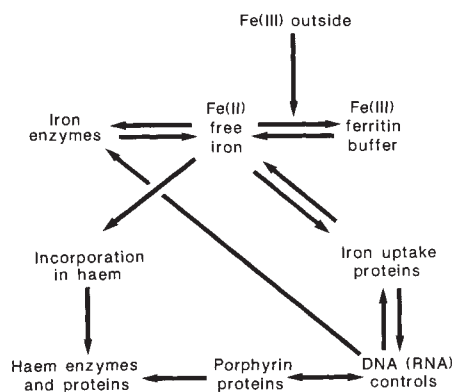


FIG. 2 The complicated interaction between iron and the controls and catalysts of a bacterial cell. Many iron enzymes are Fe_n/S_n proteins. The question posed by the findings described in this article is whether in early anaerobic life ferritin, the present-day iron store, was replaced by a mineral iron sulphide.

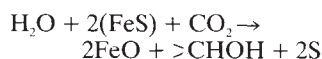
crystallization, and we would ourselves dearly like to have this control over crystallization.

The discovery of the extensive deposits of iron in a variety of bacteria leads to questions about the usefulness of such deposits, because some of the iron sulphides are not magnetic and presumably the earliest iron oxides deposited were not magnetites but were closely related to ferrihydrite. The need to store iron is related of course to the essential role of the element in a wide range of catalytic enzymes (which, in a sense, parallels the storage of calcium in bones). Iron homeostatic levels also control a whole range of enzymes essential in bioenergetics (see Fig. 2). Buffering the iron levels in aerobic cells is the mineral ferritin; but curiously hidden under this name is a variety of crystalline and amorphous iron hydroxy oxides, wrapped in somewhat similar proteins, and combined with very different amounts of phosphate depending on the particular organism concerned⁴. These ferritins are likely to be precursors of the Fe_3O_4 crystals, now known to be common in soils¹.

The occurrence of several forms of iron sulphide in sulphur bacteria² leads one to speculate as to whether sulphur bacteria have a homeostatic device based on Fe/S rather than on Fe/O solids, and whether this was the earlier form of iron store. In these organisms there is a very basic link between sulphide and iron metabolism and homeostasis. Both are also necessary to maintain the core grouping of the essential primitive electron-transfer proteins, that is Fe_n/S_n clusters where $n = 2, (3), \text{ or } 4$. The earliest energy-capture devices leading to ATP formation were based on these proteins long before the advent of dioxygen chemistry³. An Fe/S homeostatic economy for primitive life could have many other ramifications. After all pyrites (FeS_2) is half-way from sulphide to elemental sulphur, which was

one of the early metabolic end-products. The general idea of early life based on iron/sulphur chemistry is not new and has been explored and reviewed by Hartman⁶, who suggested that we should look further to see if very primitive redox systems could have used organic as well as inorganic sulphur chemistry in an alternative programme for chemical-bond energy capture before the use of ATP.

So could the iron sulphides have been a direct source of energy for early life? Electron-rich iron sulphides are not stable in water but are present in ocean vents and in some geological formations. The overall reaction of interest would be



which could be driven in part by light⁶. Did primitive life systems capture colloidal particles of iron as catalysts, in line with views of biological catalysis earlier in this century? The surfaces of iron sulphide minerals could also have been catalysts for such reactions as those now seen in Fe/S cluster proteins (for example, hydrogenases and dehydratases such as aconitase), which could conceivably be the reason for the appearance of different iron sulphides in the organisms described on pages 256 and 258. A magnetic sensor could not have been of much use in the

ARTIFICIAL INTELLIGENCE

Recognizing three dimensions

H. Christopher Longuet-Higgins

ONE of the human abilities that robot designers would most like to emulate is that of recognizing objects from their appearances. It seems fair to say that we simply do not know how the shapes of three-dimensional objects are represented in the long-term memory, how these representations are established in the first place or how they are deployed in the task of visual identification. Our ignorance is due not so much to a shortage of neurophysiological evidence as to a dearth of ideas worth testing. How could a network such as the human visual system learn to recognize particular objects after seeing them only a limited number of times, from different viewpoints and under different conditions of illumination? On page 263 of this issue¹ Poggio and Edelman propose an answer to precisely this question.

As Poggio and Edelman point out, a 3-D object gives rise to a limitless variety of 2-D images, both because of its infinite number of possible positions relative to the viewer, and because of the multiplicity of possible lighting conditions. The problem of lighting may be overcome by working with relatively invariant image features such as discontinuities in inten-

anaerobic early world.

As always in biology, when a new set of compounds is uncovered — be it a pigment such as bacteriorhodopsin, a new coenzyme such as PQQ, or minerals such as Fe_3O_4 , FeS_2 and Fe_3S_4 — we must search for the functional advantage of the material in the biological niche in which it is synthesized. The discovery of magnetic iron oxides in soil bacteria¹ is in itself peculiar, because it is hard to see the value of a navigational aid there, while the finding of iron sulphides deposited in cells gives us a new line to follow back towards the origin of life. Energy capture based on Fe/S compounds, now and perhaps before there was life, is as important as DNA in life's history. There could well be a huge variety of life in the sulphide-rich zones on Earth, perhaps holding fresh clues to help answer the question of how life began. □

R.J.P. Williams is in the Inorganic Chemistry Laboratory, University of Oxford, South Parks Road, Oxford OX1 3PN, UK.

1. Fassbinder, J.W.E., Stanjek, H. & Vail, H. *Nature* **343**, 161–163 (1990).
2. Farina, M., Esquivel, D.M.S. & de Barros, H.G.P.L. *Nature* **343**, 256–258 (1990).
3. Mann, S., Sparks, N.H.C., Frankel, R.B., Bazylinski, D.A. & Jannasch, H.W. *Nature* **343**, 258–261 (1990).
4. Mann, S., Webb, J. & Williams, R.J.P. (eds) *Biomimetalisation* (VCH, Weinheim, 1989).
5. San Pietro, A.G. (ed.) *Non-heme Iron Proteins* (Antioch, Yellow Springs, Ohio, 1965).
6. Hartman, H. *J. molec. Evol.* **4**, 359–370 (1975).

sity, and Poggio and Edelman's main concern is with the problem of variable viewpoint. One approach, widely used by robot-vision engineers, is to store in the robot's memory an explicitly 3-D representation of the object, indifferent to viewpoint. When an image appears on the robot's 'retina', its features and those of the candidate object lead to a hypothesis about the viewpoint; the system then computes the appearance of the object from the viewpoint and compares the result with the image. An unsatisfactory comparison prompts a change of hypothesis — about the viewpoint, the identity of the object or both. Not only is this process horribly expensive in computing time, it leaves unsolved the problem of how the robot is to get to know about the object in the first place. In most existing schemes this information is either supplied in advance by the user or obtained with range finders or other active sensing equipment.

Poggio and Edelman start from the hypothesis that the visual recognition of objects does *not* require that their 3-D shapes have to be explicitly represented in memory, only that the system has been exposed to enough views of a given object

to be able to recognize another such view when it sees it. What they actually do is to train their network to emit a unique 'standard' view of the object in response to the input of any one of a representative set of views. In this context the word 'view' is something of a euphemism: it signifies not an optical image but a vector whose components are the image coordinates of a few identifiable features of the object. The hypothetical 3-D objects with which Poggio and Edelman mainly deal are 'bent wire' models with two ends and four corners, so that each 2-D 'view' is a vector with 12 components altogether. Only if this vector can be approximated as a linear combination of the representative views will the network, after training, emit the standard view in response to it — a welcome demonstration of its visual competence.

Poggio and Edelman admit that the extraction of such view vectors from a set of retinal images would be a non-trivial task, involving (among other things) the solution of a multiple-correspondence problem between different images. It seems likely, moreover, that formidable book-keeping problems will arise when the appearances of a large number of objects have to be committed to visual memory. For a number of reasons, however, their work should be of interest to both theoretical and experimental psychologists.

Mathematicians working on neural nets will be reassured to know that the recently proposed approximation scheme that underlies Poggio and Edelman's system — the so-called generalized radial basis function (GRBF) scheme — works so well in such a key application. Theoreticians who are not on the circulation list for unpublished MIT research reports will be happy to see how relatively straightforward it is to implement the GRBF scheme in neural networks of a now conventional kind. They will, furthermore, be interested to learn of the recent proof by Basri and Ullman² that any orthogonal projection of a transparent polyhedron (a 3-D object all of whose vertices are visible from any direction) can be represented as a linear combination of three orthogonal projections along arbitrary directions in space — a fact that underlies a simple algorithm for determining whether a given image could or could not be an orthogonal projection of that object. Poggio and Edelman recognize their own scheme as a natural extension of Basri and Ullman's work.

On the experimental side, Poggio and Edelman make some pertinent observations about the numbers that emerge from their computational experiments. They find, for example, that the number of training views needed to achieve an acceptable recognition rate for novel views is 80 to 100 for the full viewing

sphere. This figure harmonizes nicely with Rock and DiVita's finding³ that people have trouble in recognizing a wire-frame object if it is rotated more than about 30° away from any position in which they saw it before, coupled with the fact that it takes $72 \times 30^\circ \times 30^\circ$ patches to cover the viewing sphere. Finally they point out that their GRBF recognition scheme requires the combination of 'receptive fields' by network units somewhat similar to the 'grandmother cells' beloved by some neurophysiologists. So perhaps

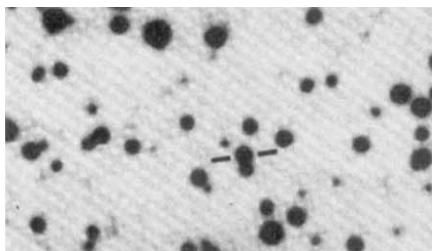
NOVA EXPLOSIONS

The long hot summer

Sumner Starrfield and R. Mark Wagner

THE explosion of a recurrent, classical or X-ray nova is one of the most violent events that can occur in a galaxy. The discovery of such an event will stimulate astronomers worldwide to observe it with many techniques and at various wavelengths. This explains the excitement, evident at a recent meeting*, generated last summer when an X-ray nova, a recurrent nova, two classical novae and the bizarre Cygnus X-3 were all found to be in outburst.

The series of outbursts began on 22 May 1989 when the All Sky Monitor on board the Japanese Ginga satellite discovered an X-ray source in the constellation Cygnus¹. Upon learning of the discovery through the International Astronomical Union (IAU) telegram network (IAU Circ. No. 4782), we immediately set out to identify



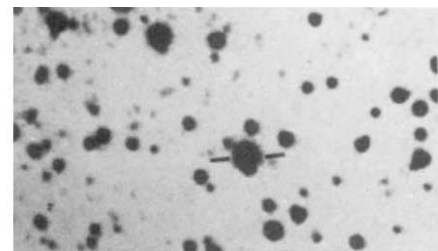
we do, after all, have brain cells that fire when and only when Grannie makes her appearance. □

H. Christopher Longuet-Higgins is in the Centre for Research on Perception and Cognition, University of Sussex, Brighton BN1 9QG, UK.

1. Poggio, T. & Edelman, S. *Nature* **343**, 263 – 266 (1990).
2. Basri, R. & Ullman, S. *Artif. Intell. Lab. Memo No. 1,152* (Artificial Intelligence Laboratory, MIT, Cambridge, 1989).
3. Rock, I. & DiVita, J. *Cognitive Psychol.* **19**, 280 – 293 (1987).

catalogued (see figure), but interstellar extinction rendered the IUE spectra blank. Within a few hours, our optical spectra confirmed that the X-ray source was identical with V404 Cyg (IAU Circ. 4783).

Our initial observations were followed rapidly by multiwavelength observations by several astronomers at infrared, radio and γ -ray wavelengths (IAU Circs 4786, 4790, 4794, 4797, 4800, 4816, 4879). For example, the radio studies of V404 Cyg, as reported by R. M. Hjellming and X. Xan (IAU Circs 4790, 4796, 4879) and at the meeting showed that the radio behaviour of this outburst was completely unlike that of the other X-ray novae. They found very short-timescale variations and quasi-periodic oscillations. The data gathered over the next few months showed that this outburst was completely different from



Negative images of V404 Cygni before (13 July 1951, from the Palomar Sky Survey; left) and after (2 June 1989, by T. J. Kreidl and S. B. Howell; right) the nova explosion of 22 May 1989.

the optical counterpart of the source. This task was complicated because the actual position lay outside the initial X-ray error box. However, B. Marsden, editor of the IAU circulars, pointed out to us the close correspondence in the X-ray position to that of a nova, V404 Cygni, last known to be in outburst in 1938, listed in the *Atlas of Galactic Novae* by Duerbeck². An electronic-mail message to A. Cassatella at the International Ultraviolet Explorer (IUE) satellite observatory in Spain alerted him to re-direct the satellite to observe V404 Cyg (the mid-afternoon Sun in Arizona left us temporarily helpless). Indeed, V404 Cyg was brighter than

the other well-studied long-period X-ray novae — A06200-00 (V616 Mon) and Cen X-4.

The outburst of V404 Cyg was soon followed by outbursts of Cygnus X-3 in June and July (IAU Circs 4798, 4817, 4826). Then in August, a recurrent nova, V745 Scorpii (IAU Circs 4820, 4821, 4822, 4825, 4826, 4844, 4853, 4885), and a classical nova, Scorpii 1989 (4836, 4838, 4839, 4840), were discovered to be in outburst. Finally, in September another classical nova, Scuti 1989, was found in outburst (IAU Circs 4861, 4862, 4865).

The discovery of V404 Cyg and V745 Sco demonstrate the need to study those outbursts that have been recorded in the past as having low amplitudes at optical

*11th North American Workshop on Cataclysmic Variables, Santa Fe, 9–13 October 1989.

frequencies with the rise from minimum to maximum being only a factor of 10^2 – 10^4 . This is in contrast to a normal classical nova in which the rise from minimum to maximum can exceed factors of 10^6 . In an X-ray nova the optical brightness may rise only 10^2 while the rise at X-ray frequencies can exceed factors of 10^4 . Interestingly, V394 Corona Astrina, V745 Sco and V404 Cyg had all been catalogued as classical novae with small-amplitude optical outbursts. It was not until they were found in outburst again that it was realized that they are unusual objects. It now seems clear that if even fragmentary data on an existing outburst indicate a small-amplitude rise, the system should be analysed and monitored for another outburst.

The spectroscopic observations of both recurrent and X-ray novae show remarkable similarities and, in addition, there was also a strong similarity between the optical spectra of V404 Cyg at maximum, of the X-ray nova A06200-00 about 2–3 weeks after maximum, and of the extraordinary object SS433. These systems all showed strong emission lines from hydrogen and neutral helium. In some of these outbursts emission lines from ionized helium can become very strong and, in some cases, this indicates non-solar abundance ratios of hydrogen to helium in the ejected material^{3,4}.

This similarity is not so surprising, because the objects have the same basic structure: a binary-star system with one large star, the secondary, that fills its 'Roche' lobe (inside which its gravity dominates gas orbits) and the other star a compact object that can be either a white dwarf, neutron star or black hole. Where the Roche lobes of the two stars touch (at the 'inner lagrangian point'), material can overflow from the secondary and reach the surface of the compact object via an accretion disk. In recurrent and classical novae, the compact star appears always to be a white dwarf, whereas in X-ray novae it is either a black hole or neutron star⁵.

The binaries' orbital periods indicate that for some recurrent novae the larger star must be close in size to a main sequence star, whereas others have much longer periods and the secondary must be much bigger in size and so must have evolved to fill its Roche lobe. T Corona Borealis and RS Ophiuchi, which have very long orbital periods, are probably recurrent novae with giant secondaries. Observations show that V745 Sco also has a giant secondary (IAU Circs 4844, 4885) and it should turn out to have a very long orbital period. In contrast, U Scorpii and V394 CrA are probably recurrent novae with evolved, but small, secondaries, because B. Schaefer (NASA/Goddard Space Flight Center) reported at the meeting that he had found orbital periods of 1.25 days for U Sco and 0.76 days for V394

CrA. X-ray novae all seem to have short periods (A06200-00 has a period of 7.3 hours and Cen X-4 has a period of 15.1 hours) and, although the period of V404 Cyg has yet to be determined, we predict that it will also be found to be short.

There are currently two competing models for the cause of the outbursts in recurrent nova systems. The first is an adaptation (by one of us, S.S., and colleagues^{6,7}) of the thermonuclear runaway theory that was originally developed for the classical nova outburst. This assumes that a layer of hydrogen-rich material is transferred from the secondary onto the white dwarf. When enough material has been accreted, the bottom of the layer reaches thermonuclear burning temperatures and an explosion results.

The second theory is that an episode of increased mass-transfer or accretion-disk instability occurs that causes a period of enhanced mass transfer onto the compact object⁸. The system then becomes bright as a result of radiated accretion energy. It is not currently possible to distinguish observationally between these two hypotheses for a particular recurrent nova. However, X-ray nova outbursts must arise from either a mass-transfer or accretion-disk instability because there is no other way of depositing the necessarily large amount of accreted material onto the neutron star to produce an outburst that lasts for months. This is also true for those systems that have black holes as the compact component.

Comparisons at the meeting of the X-ray, optical and radio data obtained at short notice during the outburst of V404 Cyg, demonstrated the importance of fast response and coordinated multiwavelength observations to our continuing understanding of both X-ray and recurrent novae. Advanced space-borne and ground-based observatories, soon to operate across the entire electromagnetic spectrum and linked by a worldwide network of computers for realtime communication, may well provide the crucial observations necessary to test models of recurrent and X-ray novae, and other astrophysical phenomena as well. □

Sumner Starrfield is at the Department of Physics and Astronomy, Arizona State University, Tempe, Arizona 85281 and R. Mark Wagner is at the Department of Astronomy, Ohio State University, Columbus, Ohio 43210, USA.

1. Kitamoto, S. *et al.* *Nature* **342**, 518–520 (1989).
2. Duerbeck, H.W. *A Reference Catalogue and Atlas of Galactic Novae* (Springer, Berlin, 1987).
3. Barlow, M.J. *et al.* *Mon. Not. R. astr. Soc.* **195**, 61–78 (1981).
4. Williams, R.E. *et al.* *Astrophys. J.* **251**, 221–229 (1981).
5. McClintock, J.E. & Remillard, R.A. *Astrophys. J.* **308**, 110–122 (1986).
6. Starrfield, S., Sparks, W.M. & Truran, J.W. *Astrophys. J.* **291**, 136–146 (1985).
7. Starrfield, S., Sparks, W.M. & Shaviv, G., *Astrophys. J.* **325**, L35–L38 (1988).
8. Webbink, R.F., Livio, M., Truran, J.W. & Orio, M. *Astrophys. J.* **314**, 653–672 (1987).

Falling metal

SNOW, says Daedalus, is one of the most interesting of solids. Its open, branched, crystalline structure gives it a very low density; it absorbs sound and insulates heat extremely well, and can be compacted into a strong structural material. In principle, any solid can be formed into a snow by mixing its vapour with air or some other inert gas, and cooling the mixture below the freezing point of the solid. The supersaturated vapour will then precipitate directly as crystals without going through the liquid phase. A growing gas-suspended crystal has great difficulty in disposing of its latent heat of condensation, and takes up an open, feathery, dendritic snowflake structure. Daedalus is now trying the process with metals.

The metal with the highest vapour pressure at its freezing point, and therefore the easiest snow former, appears to be thulium. DREADCO meteorologists are cooling mixtures of thulium vapour and argon in a pilot hot-precipitation column, and studying the resulting thulium snow under a microscope. When the process is fully understood, they will be ready to apply it to more traditional engineering metals.

Daedalus's metallic snow will be a light, fluffy, dead-black powder — maybe the blackest solid known, thanks to its micro-labyrinthine structure. Like monocrystalline metallic whiskers, its individual 'snowflakes' will be immensely strong. It should make a wonderful reinforcing agent for plastics and ceramics. And passing a current through the powder, spot welding the crystals together at their innumerable points of contact, should give a uniquely light and strong 'expanded metal'. DREADCO's 'Snometal' should revolutionize engineering.

For a start, it will absorb energy wonderfully. Snometal bumpers, traffic barriers, crash hats and safety cages will soak up any amount of punishment by the micro-deformation and crushing of their open structure. By the same token, Snometal will lend itself to all sorts of extruding and rolling and forming and pressing operations. In particular, it could be pressed into car bodies that are far lighter than normal sheet-steel ones, yet more rigid and energy absorbing. Even better, Snometal car bodies could be 'filled' with a suitable polymer. The resulting light, rigid, colourful and totally rustproof structure would last forever. Even after a collision, it could be pressed back to its original shape in a way that is impossible for fully dense steel. The steady obsolescence of all road vehicles, a major contributor to the global waste of fuel and materials, would be splendidly checked. And the pointless ritual of polishing and washing would be over for millions of resentful motorists.

David Jones

Dating sea level in caves

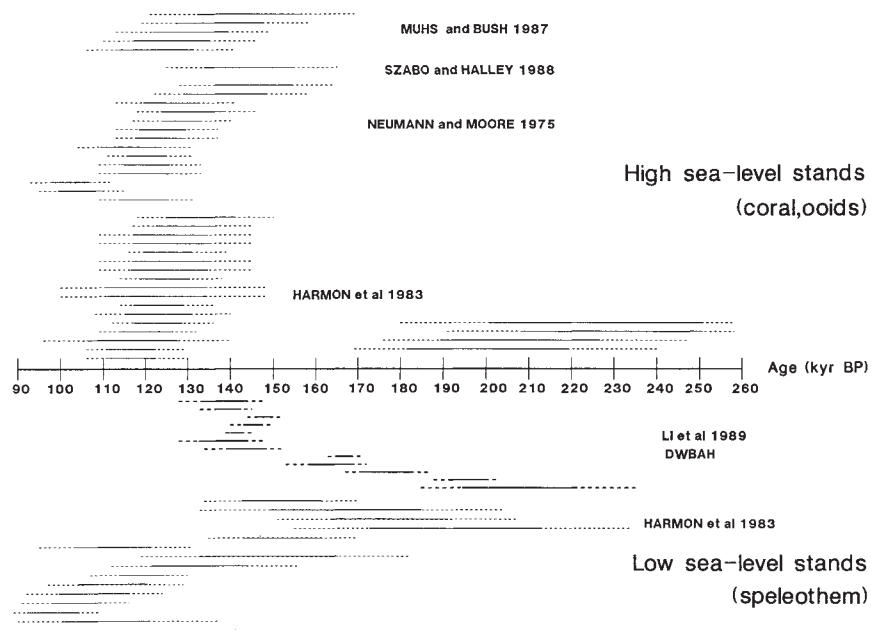
SIR—In their account of their dating of the calcite deposits (known as speleothem) from a cave in the Bahamas, Li *et al.*¹, refer in Table 1b to “equivalent samples” of the DWBAH speleothem. But inspection of Fig. 1 shows that some of the samples compared are not stratigraphically equivalent. For example, alpha-count-dated sample MB is coupled with mass-spectrometrically dated sample A1 in the table; yet, as is clear from Fig. 1, sample MB is at least twice as thick and contains younger material than sample A1. No wonder that it dates ~40 kyr younger than A1. Similarly, alpha-dated sample TT, which is coupled with mass-spectrometrically dated sample 18, contains flowstone stratigraphically older than in sample 18; and not surprisingly has a much older date. Where the mass-spectrometrically dated sample is from a stratigraphic interval near the middle of the thicker alpha-dated samples, comparable ages are obtained in two of three cases. The authors point out (Fig. 1 legend) that about ten times as much sample is needed for alpha-count as for mass-spectrometric dating. Nevertheless, a meaningful comparison of the alpha-count and mass-spectrometric ages requires samples from identical stratigraphic levels, a procedure not followed.

Li *et al.*¹ claim (their Fig. 3) that sea level was below about -12 m (the depth of speleothem DWBAH) between 135 and 215 kyr before present (BP), and that their record is generally “in agreement with the high-sea-stand chronology inferred from oxygen isotope stratigraphy of oceanic foraminiferal cores”. Unfortunately, they fail to cite published evidence to the contrary. R. S. Harmon *et al.*² have used α -counting dates of submerged speleothem and emerged corals to show that sea level stood between the modern level and -6 m about 195–210 kyr ago. Moreover, the high stand shown in ref. 2 falls beneath the highest $\delta^{18}\text{O}$ peak of marine isotope stage 7 reproduced by Li *et al.* (their Fig. 3) at a time when they indicate sea level as below -12 m. Figure 6 of ref. 2 also shows a short-lived sea-level high (shallower than -7 m) at about 145–150 kyr BP, when Li *et al.* indicate that sea level stood below -12 m, and possibly³ deeper than -40 m.

That sea level may have reached or exceeded modern levels by 145 kyr BP, in contradiction to the marine $\delta^{18}\text{O}$ chronology and to the data of ref. 1, is supported by other studies from the Bahamas, and from adjacent southern Florida. Alpha-count dating of corals and ooids from the Bahamas indicates that the sea-level history of this relatively stable platform is complex, with one or more high sea-level stands between 100 and 145 kyr BP^{4,5}. For example, Neumann and Moore⁴ dated two

Bahamian corals at 146 ± 9 kyr BP and 140 ± 9 kyr BP; these corals were >95 per cent aragonite. And the Key Largo Limestone of south Florida was dated at 139 (+19/-14) kyr BP (an average taken from the data of 13 participating laboratories)

resolution obtainable by mass spectrometry in a time range where the resolution of alpha-count dates is poor (below Hiatus 1) and in pinpointing events (terminations). The alpha-count dates in question have high error because of low uranium contents (0.09 p.p.m. on average) and low yields (32–80% for U, and 8–65% for Th). Winograd also questions the number



Dates on corals and ooids indicate high sea-level stands; those on drowned speleothem indicate low sea-level stands. —, 1 σ error; - - - - - , 2 σ error.

during the first uranium-series intercomparison project⁶; the coral specimen used was 90 per cent aragonite. Recently, Szabo and Halley⁷ obtained an age of 145 ± 10 kyr for the Key Largo Limestone using a coral containing >97 per cent aragonite.

Are we to assume that the dates in refs 2 and 4–7, indicating a high sea-level stand at around 140–150 kyr BP, are all 10–20 kyr too old? Or might there be an undetected hiatus in speleothem DWBAH between 145 and 165 kyr BP? No ages are provided by Li *et al.*¹ for this time interval, which corresponds to a 15 mm thickness of DWBAH (their Fig. 2). No other interval this thick is undated in DWBAH.

Speleothem DWBAH provides the authors with an excellent opportunity to address explicitly the decade-old debate of whether the last interglacial was marked by a single or a double rise in sea level, I urge them, therefore, to address these matters.

ISAAC J. WINOGRAD

US Geological Survey,
National Center (432),
Reston, Virginia 22092, USA

LUNDBERG *ET AL.* REPLY—Winograd is right in saying that the alpha-count and mass spectrometric dates in Table 1b are not directly comparable. They were not intended to be; they show the superior

and ages of high sea-level stands during isotope stages 7 and 5e. Although it is customary to quote α -counting dates with only 1 σ error (the 68% confidence interval), there is a 32% probability that the true value is not in this range. If one looks instead at the 2 σ errors (95% confidence interval), many apparent conflicts with the mass-spectrometry data disappear (see figure).

Harmon *et al.*² suggest that sea level rose to about +2 m between 210 and 190 kyr BP. In our DWBAH sample the first mass-spectrometric date after the stage 7a/7c recession (sample A1) is 237–186 kyr BP (2 σ range). The equivalent stalagmite date in ref. 2 is 235–155 kyr (2 σ range). Their four coral dates for the previous high sea-level stand range from 282 to 168 kyr; their coral and stalagmite dates overlap each other and both of the stage 7 hiatuses (1 and 2) resolved by mass spectrometry. Note that the date of isotope stage 7a itself has been only approximated (by orbital tuning), and that Chappell and Shackleton⁸ modified their sea-level curve to fit this expected rise: their original (pre-tuned) dates at ~220 and 235 kyr BP coincide with hiatuses 2 and 1 (at around 220–212 and 235–230 kyr BP) from DWBAH.

The marine isotope record gives no reason to suspect a rise of sea level to modern heights at ~145 kyr BP, although

several α -counting dates, considered in isolation, might. DWBAH shows that the sea rose after 149–129 kyr BP. Harmon *et al.*² place cessation of stalagmite growth at 156–112, 171–135 and 182–118 kyr BP. Their coral dates range from 150 to 106 kyr BP; those of ref. 4, 164 to 93 kyr BP ("with no evidence of any discrete, distinct peaks that might be interpreted as specific sea-level maxima"), and ref. 7, 165 to 125 kyr BP. Dates on ooids and peloids⁵ range from 169 to 105 kyr BP. These ages cannot be resolved into two

populations. There is as yet no evidence for more than one major sea-level rise at stage 5e; under magnification, DWBAH shows continuous deposition between hiatus 2 and hiatus 3.

DWBAH indicates that at ~ 12 m below modern sea level, deposition occurred until ~ 134 kyr BP or later, and did not resume until ~ 110 kyr BP. Any oscillations that may have occurred above -10 m (as shown in ref. 8) would thus not be recorded in DWBAH.

J. LUNDBERG*

D. C. FORD*

H. P. SCHWARCZ†

A. P. DICKIN†

W.-X. LI†

Departments of *Geography and

†Geology,

McMaster University,

Hamilton, Ontario,

Canada L8S 4K1

1. W.-X. Li *et al.* *Nature* **339**, 534–536 (1989).
2. Harmon, R. S. *et al.* *Paleogeogr. Paleoclimat. Paleocol.* **44**, 41–70 (1983).
3. Gascoyne, M. *et al.* *Science* **205**, 806–808 (1979).
4. Neumann, A. C. & Moore, W. S. *Quat. Res.* **5**, 215–224 (1975).
5. Muhs, D. R. & Bush, C. A. *Prog. Geol. Soc. Am. (abstr.)* Vol 19, No. 17, 780 (1987).
6. Harmon, R. S. *et al.* *Geology* **7**, 405–409 (1979).
7. Szabo, B. J. & Halley, R. B. *Prog. Tenth Biennial Mtg. Am. Quat. Assoc. (abstr.)* 154 (1988).
8. Chappell, J. & Shackleton, N. J. *Nature* **324**, 137 (1986).

Disposable filters may damage your cells

SIR—Cultured cells are widely and increasingly used in biomedical research. In my own case, I study the mechanism of secretion using primary cultures of bovine adrenal medullary cells. As anyone with experience of tissue cultures will know, there are times when things "don't go right" with cell cultures.

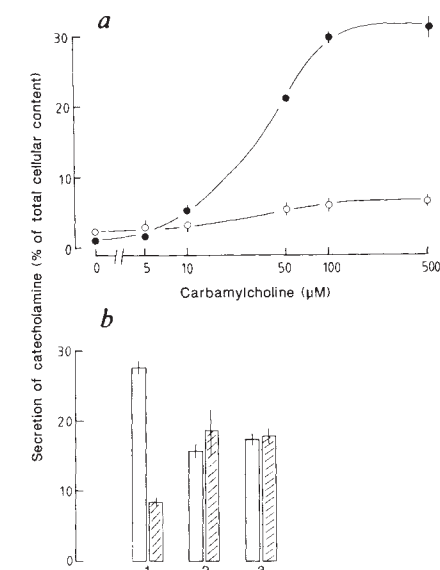
Recently I had one such experience, isolating and plating out chromaffin cells

lem was narrowed down to the disposable syringe filters used for sterilizing the solutions.

The filters were 0.2 μ Flowpore Disposable filters D26 series, bought in boxes of 50. Solutions used for washing and disaggregating the cells were sterilized by passage through these disposable filters. Furthermore, culture medium containing various additives were also filtered. When

The effect of filtrate on cultured bovine chromaffin cells. Cells were isolated and cultured as described (Knight, D. E. *FEBS Lett* **207**, 222–226; 1986). After 3 days culture, the medium on the cells was replaced by fresh medium that had or had not been passed once through Flow Pore D26 0.2 μ syringe filters. Cells were then washed twice in physiological saline and challenged to secrete with; a, various levels of carbamylcholine or b, (1) 100 μ M carbamylcholine, (2) 150 mM K^+ , or (3) a 10 μ M Ca^{2+} challenge using CaEGTA buffers, after first rendering the cells leaky for 6 min in potassium glutamate based medium at 0.01 μ M Ca^{2+} and containing 10 μ M digitonin. The catecholamine secreted over 15 min in response to each of these challenges is shown. a, Cells incubated for 18 h in 1 ml per well of filtered culture medium (50 ml culture medium passed once through 5 disposable filters) ($\circ$). Control cells incubated for the 18 h in unfiltered culture medium ($\bullet$). Data are means of 3 determinations $\pm$ s.d. b, Cells incubated for 3 days in 1 ml per well filtered culture medium (20 ml culture medium passed once through 5 filters (different batch from those in a)) (hatched bars). Control cells incubated for the 3 days in unfiltered medium (open bars). Data are means of 6 determinations $\pm$ s.d.

for nearly three months only to find that, within 24 hours of isolation, vacuoles appeared in the cells, many did not attach very well to the plates and those that did responded very poorly to a secretagogue. Their conditions deteriorated steadily over the next few days. After eliminating many possible factors — culture medium, plastics, chemicals used for cell isolation, condition of cattle, and so on — the prob-



cells were cultured without using these filters, they appeared healthy and secreted catecholamine. Addition to these cells of culture medium passed through the filters halved, within 24 hours, the amount of catecholamine in the culture wells and almost completely abolished the secretory response (a in figure).

The toxicity of the filters was found to vary between batches and sometimes

became apparent only after several days of culture. For example, b in the Fig. shows the effect of incubating cells for 3 days in the filtrate processed by one such batch of filters. Here the secretory response to carbamylcholine was inhibited by over 50% although the catecholamine content of the cells was unaltered, but secretion was not inhibited when the cells were challenged with high concentration extracellular potassium, which opens voltage dependent calcium channels, or when buffered calcium was introduced directly into the cell after first permeabilizing the membrane with digitonin. These data suggest that the extent of toxicity varies with the stimulus, first affecting receptor mediated responses.

Inquiries by the suppliers, Flow Laboratories Ltd, to the manufacturers Sartorius, revealed that during assembly filters are wetted with polyglycoethers.

Sartorius have now said that they are changing the wetting procedures of the filter assembly in view of the possible toxic effect of polyglycoethers.

Flow Laboratories Ltd are in agreement that the users of these disposable syringe filters should be made aware of possible toxic effects on cell cultures. They suggest that to alleviate the problem with the existing filters, 10 ml of warm distilled water be passed through the filter, followed by a few mls of culture media, before the filter is used for filtration of media destined for cultural purposes.

D. E. KNIGHT

Division of Biomedical Sciences,
King's College,
Strand,
London WC2 R2LS, UK.

NF- κ B and HIV

SIR—Griffin *et al.* suggest¹ that the transcription factor NF- κ B is activated during differentiation of promonocytic stem cells and that this contributes to the induction of the human immunodeficiency virus (HIV) in latently infected monocytes. Their conclusion is based solely on experimental work with the human promonocytic cell line U937, which is not identical to normal promonocytic stem cells. This approach raises difficulties because transcriptional regulation of individual genes in normal tissue has been shown to differ substantially from regulation in corresponding cell lines². To validate this hypothesis on the basis of observations with U937 cells, it is necessary to study κ B binding and transcriptional activity in normal promonocyte and monocyte cells.

Although Griffin *et al.* have correlated HIV gene activation with the appearance of κ B-binding activity following induction of U937 cells, previously published experiments^{3,4} have demonstrated κ B-binding activity in non-induced cells. Both protein binding and *in vitro* transcription assays

have disclosed that nuclear factors from non-induced U937 cells bind to κ B sequences in the HIV-LTR and stimulate transcription at least ten-fold¹. In fact, the U937 cell line appears to be composed of at least two phenotypically different sublines, one of which expressed NF- κ B in an inducible manner and a second in which NF- κ B is constitutively expressed (anonymous referee, unpublished observations).

We suggest, therefore, that the correlation of NF- κ B induction and transcriptional activation of the HIV-LTR with monocyte differentiation shown by Griffin *et al.* needs to be substantiated in normal promonocytes before any firm conclusions about monocyte differentiation, κ B binding activity and induction of HIV gene expression can be drawn.

LOTHAR HENNIGHAUSEN

PRISCILLA A. FURTH

National Institutes of Health,
Bethesda, Maryland 20892,
USA

GRIFFIN *ET AL.* REPLY—Hennighausen and Furth raise two issues regarding NF- κ B binding in the monocyte lineage. We acknowledged that phenotypic differences among cell lines are of great importance; we carefully detailed the phenotype of our monocyte-like tumour cell lines. Our findings in the U937 line were also confirmed in the independent myelomonocytic cell line HL-60, representative of an earlier stem cell, which, when induced to differentiate to the monocyte lineage, also acquired NF- κ B binding activity. So finding that NF- κ B binding can be detected in some U937 sublines is thus not unexpected; it would be of interest to know the phenotypic characteristics of this cell line.

It is also important that we confirmed our findings in normal cells, showing that monocytes and macrophages of mouse and man contain NF- κ B (see ref. 1, Fig. 2b). Thus there is no obvious discrepancy between mature U937, HL-60 or other macrophage leukaemia cells in tissue culture and normal monocytes and macrophages *in vivo*. We agree with Hennighausen and Furth that it would also be of interest to analyse NF- κ B binding in promonocytic stem cells, but these are rare and not amenable at present to further study.

Several factors can affect the detection of NF- κ B binding in cultured cells. Cells not maintained in log phase, or which are contaminated by mycoplasma, may acquire NF- κ B binding activity, possibly

by differentiation. Such conditions could contribute to the detection of NF- κ B binding in the uninduced U937 cells of Lubon *et al.*⁴ and could also explain their observation of NF- κ B binding in uninduced HeLa cells, reported previously not to contain this factor^{5,6}. Another study has reported findings similar to our own⁷. The degree of DNase protection seen for U937 by Wu *et al.* was low, and its NF- κ B binding activity increased markedly on PMA stimulation, consistent with our observations.

Further studies of normal haematopoietic cells will undoubtedly help to validate our model based on studies of leukaemia lines and normal monocytes and macrophages. Several studies have indicated that HIV infects hematopoietic progeni-

tors^{7,8}. Further studies of the role of NF- κ B in normal promonocyte differentiation and HIV infection will await more sensitive NF- κ B probes and the ability to maintain such promonocytic stem cells in continuous culture.

GEORGE GRIFFIN

KWANYEE LEUNG

THOMAS M. FOLKS

STEVEN KUNKEL

GARY J. NABEL

Medical Science Research

Building 1,

Room 4510,

University of Michigan,

Howard Hughes Medical Institute,

Ann Arbor,

Michigan 48109-0650,

USA

A chink in HIV's armour?

SIR—Elegant work has established that the human immunodeficiency virus (HIV) infects susceptible cells following interactions between the viral envelope glycoprotein (gp120) and a cell-surface receptor (CD4) (refs 1,2). However, other factors must play a role to explain, for example, why mouse cell lines expressing human CD4 on their cell surfaces are not susceptible to infection. In addition, neutralizing anti-bodies to the V3 loop of gp120 prevent infection even though they do not inhibit binding to CD4. A recent paper by Hattori *et al.*³ and results emerging from our own laboratory may shed some light on these other factors.

During the development at Celltech of a process for the production of recombinant HIV-1 gp120 from Chinese hamster ovary cells for the MRC Aids Directed Programme, we have identified a specific proteolytic cleavage event of the gp120 molecule under certain cell culture conditions. Cleavage becomes apparent only after SDS-PAGE of the purified protein under reducing conditions, indicating that the two cleavage products, relative molecular mass 70,000 (70K) and 50K, are held together by a reducible disulphide bridge. Amino-terminal sequences analysis of the 50K fragment indicated that cleavage occurs between residues 315 and 316 of the intact protein. This corresponds to the arginine and alanine residues at the tip of the V3 loop, a region previously demonstrated to be one of the major sites on gp120 able to elicit neutralizing antibodies⁴. The cleaved gp120 is still able to bind CD4 with an affinity similar to that of uncleaved material, but neutralizing antibodies directed at the V3 loop are unable to bind cleaved gp120.

Hattori *et al.* have recently shown that trypsin, a new Kunitz-type protease inhibitor purified from rat mast cells, inhibits the formation of syncytia in HIV-1 infected cultures of CD4 T cells⁵. As trypsin has a similar sequence to the V3

loop of gp120 and as a synthetic peptide corresponding to the loop both inhibit protease activity and prevents syncytia formation, it may be that cleavage of this loop is essential for viral infection. Interestingly, it is predicted that the reactive site of trypsin is the same as the arginine-alanine site that we have found is cleaved during gp120 production. Experiments are now in progress at Celltech to determine whether this specific cleavage of the V3 loop is a prerequisite for viral infection.

The requirement for cleavage in the V3 loop may explain the inhibitory effect of neutralizing antibodies which bind this region of the protein. Presumably, the cleavage is required to bring about a conformational change necessary for the fusion of viral and cell membranes that follows binding of virus to CD4. Neutralizing antibodies may either prevent cleavage of the protein or block the conformational change that occurs upon cleavage and in this way abolish infectivity. Should murine fibroblasts lack the necessary protease activity, it would explain why they are not susceptible to HIV infection even after transfection with human CD4.

Clearly these observations indicate another possible chink in the HIV-1 armour which could lead to therapeutics for the control of AIDS, but the significance of the gp120 cleavage we here observed still needs to be established.

P. E. STEPHENS

G. CLEMENTS

G. T. YARRANTON

Celltech Ltd,

216 Bath Road, Slough, UK.

J. MOORE

Institute of Cancer Research,
Fulham Road,
London SW3 6JB, UK.

1. Daigleish, A. G. *et al.* *Nature* **312**, 763–767 (1984).

2. Kkatzmann, D. *et al.* *Nature* **312**, 767–768 (1984).

3. Hattori, T. *et al.* *FEBS Letts* **248**, 48–52 (1989).

4. Rusche, J. R. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **85**, 3198–3202 (1989).

1. Griffin, G. E. *et al.* *Nature* **339**, 70–73 (1989).

2. Swift, G. H. *et al.* *Genes Dev.* **3**, 687–698 (1989).

3. Wu, F. *et al.* *J. Virol.* **62**, 218–225 (1988).

4. Lubon, H. *et al.* *AIDS Res. human Retrovir.* **4**, 381–391 (1988).

5. Sen R. & Baltimore, D. *Cell* **47**, 921–928 (1986).

6. Baldwin A. S. & Sharp, P. A. *Proc. natn. Acad. Sci. U.S.A.* **85**, 723–727 (1988).

7. Donahue *et al.* *Nature* **326**, 200–203 (1987).

8. Folks, T. M. *et al.* *Science* **242**, 919–922 (1988).

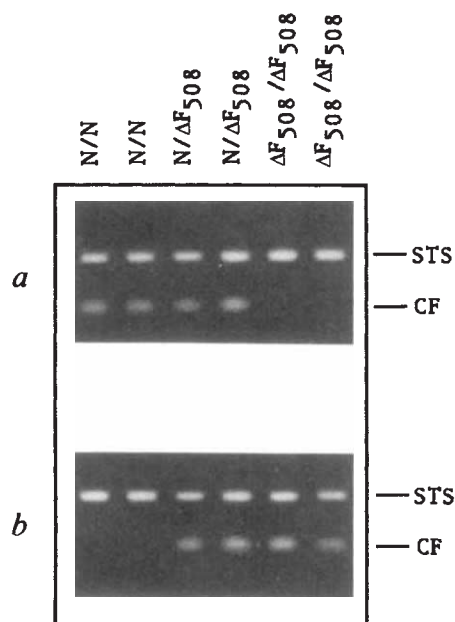
PCR test for cystic fibrosis deletion

SIR—Kerem *et al.*¹ recently reported that approximately 70 per cent of the mutations in cystic fibrosis (CF) patients correspond to a specific deletion of 3 base pairs (bp) at amino-acid position 508 (ΔF_{508}) of the putative product of the *CF*

results of allele-specific PCR amplification of DNA from six individuals who had been previously characterized using the allele specific oligonucleotide (ASO) technique (ref. 2; and A. L. Beaudet, personal communication). Two separate

FIG. 1 Ethidium-bromide stained agarose gel electrophoresis of PCR products showing discrimination of normal and ΔF_{508} alleles. N/N, N/ ΔF_{508} and ΔF_{508} correspond to homozygous normal, heterozygous and homozygous ΔF_{508} individuals, respectively. In *a*, PCR was performed with primers C16D (ref. 2) and 1468 (5'-GGCACCATTAAAGAAATATCATC-TT-3'), corresponding to the wild-type allele. In *b*, PCR was performed using primers C16D and 1469 (5'-GGCACCATTAAAGAAATATCATC-GG-3'), corresponding to the ΔF_{508} deletion allele. The steroid sulphatase (STS) gene primers 908 (5'-GGCCTAGAAGAAGGTTGAAGG-TCCG-3') and 909 (5'-AAGAGGTTGGATGA-GATGGGCATAC-3') were included in each reaction as a positive control for amplification efficiency (292-bp product). PCR (performed according to Saiki *et al.*³). Purified genomic DNA (500 ng), 30 pmol of each primer, 2.5 units of Taq polymerase and 2 mM $MgCl_2$ and 200 μM dNTP were used in each 100 μl reaction. After an initial denaturing step at 95 °C for 5 min, 30 cycles were performed, including a 45s denaturing step at 95 °C, an annealing step at 60 °C and a 1 min extension step at 72 °C. The last cycle was followed by a 7 min step at 72 °C. Aliquots of each reaction (15 μl) were electrophoresed on an ethidium bromide stained standard agarose gel (1.3% in Tris-borate buffer) and run for 30 min at 50 mA. The gel was photographed on an ultraviolet transilluminator.

gene. To detect this deletion we have developed the two simple polymerase chain reaction (PCR) tests described here.



Using the available *CF* cDNA sequence information², we designed allele-specific oligonucleotide primers, corresponding to the wild type and F_{508} alleles, and then determined the conditions that enable specific amplification of each of the two alleles by PCR^{3,4}. Figure 1 shows the re-

reactions were performed for each individual using either the wild-type primer (Fig. 1a) or the mutant primer (Fig. 1b), FIG. 2 Simultaneous of in combination with amplification of the C16D oligonucleotide² as the reverse primer². The gel, stained with ethidium bromide, clearly shows that in the lanes corresponding to normal homozygous individuals, amplification of the PCR product of the expected size (79 bp) occurred only when the wild-type primer was present. In the samples from ΔF_{508} deletion homozygotes, the PCR product (76 bp) is visible only in the presence of the mutant primers. In the heterozygotes both the wild-type and the ΔF_{508} primers generated the expected product. As an internal control for the efficiency of amplification, two primers derived from the X-linked steroid sulphatase (STS) gene⁵ were included in each reaction.

Figure 2 shows the results obtained

when the same primers were used in a competitive oligonucleotide priming reaction⁴. Both allele-specific primers were included in a single PCR and the primer incorporated was identified by the radioactively labelled the 5' terminus. In each case the radioactivity was predominantly incorporated only when the perfectly matched labelled primer was present. The ratio of the radioactive signal from the incorporation of correct-match primer to mismatched primer was approximately 50 to 1. We are at present adapting this to the use of allele-specific fluorescence-tagged primers for single-tube detection⁶.

The identification of suitable PCR primer sequences and reaction conditions for the discrimination of ΔF_{508} alleles greatly simplifies PCR diagnosis of CF. It is now possible to identify the genotype of individuals by direct visualization of an ethidium bromide stained gel, without subsequent blotting, ASO hybridization, or washing and exposure.

As the ΔF_{508} deletion is found in the large majority of CF chromosomes (68 per cent)² this test may be favoured not only for prenatal and carrier diagnosis in CF families but also for extensive carrier screening in the population.

*[†] ANDREA BALLABIO

* RICHARD A. GIBBS

*[†] C. THOMAS CASKEY

* Howard Hughes Medical Institute, and [†] Institute for Molecular Genetics, Baylor College of Medicine, Houston, Texas 77030, USA

1. Kerem, B. *et al.* *Science* **245**, 1073–1080 (1989).
2. Riordan, J.R. *et al.* *Science* **245**, 1066–1073 (1989).
3. Saiki, R.K. *et al.* *Science* **239**, 487–491 (1988).
4. Gibbs, R.A., Nguyen, P.N. & Caskey, C.T. *Nucleic Acids Res.* **17**, 2437–2448 (1989).
5. Ballabio, A. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **84**, 4519–4523 (1987).
6. Chehab, F.F. *J. Cell Biochem. Suppl.* **13E**, 278 (1989).

A craving for wax

SIR—I have discovered accidentally that our Siamese cat greatly enjoyed the taste of human earwax. I dismissed this as a curiosity until I found that our second Siamese cat also liked it. In fact, the second cat leaps on the bed in the morning hoping to be offered some. I mentioned this odd behaviour to three other people and have learned from them that their cats also liked the wax. I'd always thought that earwax tasted very bitter (as a deterrent to insects getting into your ears), so I find the cats' reaction hard to understand. Does any of your readers have an explanation? I wondered if it might be a means to induce cats to groom their kittens.

THOMAS ARNY

Departments of Physics and Astronomy, University of Massachusetts, Amherst, Massachusetts 01003, USA.

A brave new world?

J. S. Jones

Evolutionary Studies: A Centenary Celebration of the Life of Julian Huxley. Edited by G. A. Harrison and M. Keynes. Macmillan (in association with The Eugenics Society), London: 1989. Pp.256. £35.

JULIAN Huxley was one of the last of Britain's establishment biologists, and in this volume members of the biological establishment combine with others to commemorate the centenary of his birth. There are two portraits of him here; in the lap of his grandfather, Thomas Henry Huxley — Darwin's bulldog — and seated with his utopian brother Aldous. Two themes recur throughout his life: a determination to defend natural selection and an unapologetic readiness to apply evolutionary principles to the improvement of the human race.

Sir Andrew Huxley outlines his half-brother's background and career — which was extraordinary enough: a thousand publications, a professor at the age of 26, first head of the United Nations Educational, Social and Cultural Organisation (UNESCO), and among the founders of the Nature Conservancy and the World Wildlife Fund. In 1929 Julian collaborated with H. G. Wells and his son on *The Science of Life*, an enormously successful popular account of biology. The books he published in the next five years included *Birdwatching and Bird Behaviour*, *Ants*, *Africa View*, *Problems of Relative Growth*, *What Dare I Think?*, *A Scientist among the Soviets*, *The Captive Shrew and other Poems of a Biologist*, *The Elements of Experimental Embryology* and *We Europeans*, a *Survey of 'Racial' Problems*.

This breadth of interest was reflected in his research, and there is no doubt that Julian Huxley was a leading biologist of pre-war days, carrying out some of the first systematic studies of animal behaviour, of the genes controlling development and cell recognition, and of the evolution of shape and size. Huxley helped to formulate the language of biology. Cline, allometry, ritualization, and the modern synthesis itself are all terms coined by him. His belief in the overwhelming importance of selection led to some excesses in later years, and few would now agree that natural selection is always a force for progress and a "power that makes for righteousness" in human behaviour. Loyalty is not a convincing support for any theory, and Darwin is better defended by bulldogs than by lapdogs.

Huxley's biological work is brought up to date here in chapters on molecular evolution, on ethology, palaeontology, human evolution, anthropology and relative growth. There is a personal memoir by E. B. Ford, and an attempt by Patrick



T. H. Huxley with his grandson, Julian, in 1895.

Bateson to explore what evolutionary theory might or might not be able to tell us about ethics. With the exception of a balanced preface by G. A. Harrison, one of the editors of the book, there is little mention of the persistent controversy about the importance or otherwise of natural selection in evolutionary fields as different as molecular genetics and animal behaviour. As is perhaps inevitable, the ritual of commemoration involves genuflections almost as stereotyped as those of the great crested grebe: Huxley's work is described as meticulous, acute, careful, brilliant, beautiful, painstaking, illustrious and seminal.

No doubt this is true: but to read his 1962 paper "Eugenics in Evolutionary Perspective" is to realize that he could also be foolish and indiscriminating. It might — just — have been acceptable to write in 1931 that unemployment pay should be granted only to those willing to have no more children and that "infringement of this order could probably be met by a

short period of segregation in a labour camp", but the knowledge of just where that particular utopia ended up was enough to instil a certain humility into most of those who thought that society could be arranged on biological principles. Not so Huxley: his biology master at Eton noted a "tendency to think himself infallible", and less than 20 years after the war, he was blithely suggesting that social-problem groups should be obliged to practice compulsory birth control, and that human races must have considerable genetic differences in temperament and mental capacity.

Huxley shared with many of his scientific contemporaries those highly heritable human characteristics of wealth, education and social position. The agenda of eugenics from Plato to Galton and beyond has always been the survival of the richest, and it is in discussing positive eugenics that Huxley was at his most enthusiastic. He was keen on the idea, suggested to him by Sir Cyril Burt, that some types of high intelligence are determined by single genes, and that breeding from these rare individuals might do a great deal to improve the genetic constitution of humankind. Although he refused to make the appropriate deposit in an American sperm bank, he strongly supported the idea of EID (eugenic insemination by donor).

Julian Huxley was perhaps the last biologist to take himself seriously — or at least to expect that he would be taken seriously by those with the political power to implement his ideas. It is one of the surprising facts of eugenics

that as the means of reaching a genetical utopia get closer, the interest in achieving it has almost disappeared. The Laboratory for National Eugenics at University College London changed its name to the Galton Laboratory long ago, and the (quite separate) Eugenics Society, publisher of this book, has just altered its own title to the Galton Institute. In Britain, only a few millionaires, physicists, Conservative politicians and members of other fringe groups now express any concern about their eugenic duty to generations yet unborn. The explosion of knowledge about human genetics is now directed almost exclusively to improving the lot of individuals or of families rather than that of humanity. Perhaps this change in emphasis arises from a realization that biology is a lot more complicated than we ever thought, but perhaps it just shows that biologists are a lot poorer than they used to be. □

J. S. Jones is in the Galton Laboratory, University College London, London NW1 2HE, UK.

Orbital update

Patrick Fowler

Methods of Molecular Quantum Mechanics, 2nd edn. By R. McWeeny. *Academic*: 1989. Pp. 573. £65, \$130.

THEORETICAL chemistry is a peculiar subject. It is based on an equation that can hardly ever be solved. It deals in chemical concepts and qualitative rationalizations and yet its practitioners are amongst the biggest users wherever computer time and disk space are to be had. The new edition of a classic textbook offers a useful chance to see just how this odd activity has been changing. "McWeeny and Sutcliffe" has lost an author and gained several hundred pages. It remains a beautifully clear exposition of quantum chemistry written for the graduate student but useful for more experienced researchers, with newly added and sometimes tough problems at the end of each chapter. Quite deliberately, it concentrates on the conceptual and mathematical development of the subject and touches only lightly on computational aspects. In content and emphasis it is significantly different from the first edition which was issued about 20 years ago.

Since 1969 there have been several shifts in the subject. The enormous growth in computer power has, of course, extended the horizon of what is possible, but methods are also changing rapidly. Techniques and language lifted from theoretical nuclear and solid-state physics are beginning to make an impact on the everyday practice of molecular physics. Second quantization, Greens functions, propagators and diagrammatic techniques are becoming accepted as practical tools and not just ways to restate conventional results in what has sometimes seemed to the uninitiated to be wilfully esoteric form. The new edition contains an elementary account of diagrams which (almost) convinced me, at least, of their usefulness. Many-body perturbation treatments of correlation energy seem intuitively appealing and yet fail to deliver quantitative results in so many contexts that it is interesting to speculate whether they will still be prominent in textbooks of 20 years hence.

Since the early days of quantum mechanics the controversy between proponents of molecular-orbital (MO) and valence-bond (VB) theories has afforded good, clean, knockabout fun for the disinterested spectator. Because these theories start from the same equation, and converge in the limit to the same result, it is sometimes hard to see why they can have aroused such passions over the years. As used to be well known to undergraduate chemists, VB theory aims to construct

wavefunctions in which all possible chemical bonds are represented in terms of spin-pairing of the electrons in atomic valence orbitals. This inevitably leads to formidable practical difficulties with non-orthogonal orbitals, calculations that grow factorially with the number of electrons, and embarrassing 'ionic' and 'charge-transfer' contributions in the descriptions of ordinary covalent molecules. The basic MO approach is mathematically simpler and physically more appealing to many with its description of orbitals which extend over the whole nuclear framework and give an obvious rationalization of electron delocalization in π -systems and electron-deficient clusters. MO theory is much more readily translated into workable computer algorithms and came to eclipse VB both in calculation and in the descriptive language of theoretical, inorganic and eventually organic chemists. But in the pursuit of ever greater accuracy, MO calculations moved beyond the single-determinant self-consistent-field wavefunction to use vast configuration expansions which are almost as difficult to handle as giant VB functions and which rapidly lose physical interpretation in a welter of small energy terms. At the same time technical developments in VB theory mean that calculations on slightly larger molecules are now feasible and VB is enjoying something of a revival. The

controversy is not dead yet. The new edition of *Molecular Quantum Mechanics* gives more space to VB and an extensive discussion of modern approaches to the configuration-interaction problem.

Quantum chemistry used to deal exclusively with questions of electronic structure and valence. Nowadays, prompted partly by new experiments and partly by expanded computational possibilities, the study of more general molecular properties is on the increase. Electric and magnetic properties of single molecules are intimately linked to electronic structure, give information on intermolecular forces and properties of materials in bulk, and are probed by numerous sophisticated spectroscopic techniques. This book gives an excellent introduction to the theory of static and, in a new chapter, time- and frequency-dependent response of molecules to perturbation by fields and other molecules. For practical purposes, this would need to be supplemented by a discussion of multipole moments and multipole expansions which, mathematically unsatisfactory though they may be, are used in most current calculations and theoretical treatments of intermolecular forces. All in all, this is an excellent new version of an important textbook. □

Patrick Fowler is in the Department of Chemistry, Exeter University, Exeter EX4 4QD, UK.



The African zebras and Eurasian pygmy mouse, above and right, are reproduced from *Grzimek's Encyclopedia of Mammals*. This five volume set was produced by B. Grzimek in collaboration with more than 200 naturalists, zoologists, biologists and ecologists, and is a comprehensive study of animals in their natural environments. The volumes are beautifully illustrated, containing more than 3,000 colour photographs. Published by McGraw-Hill, price \$500, £325.



Looking into it

Joseph Silk

The Fifth Essence: The Search for Dark Matter in the Universe. By Lawrence M. Krauss. *Basic Books*: 1989. 342 pp. \$19.95. Distributed in Britain by *Hutchinson/Radius*, £14.95.

Invisible Matter and the Fate of the Universe. By Barry Parker. *Plenum*: 1989. 297 pp. \$28.20, £17.63.

DARK matter is cosmology's paradigm for the 1990s. Depending on who does the book-keeping, 90 per cent, or even 99 per cent, of the Universe has resolutely resisted all attempts by astronomers to detect it. One view is that the Newton-Einstein theory of gravity, which has worked so well over the scale of the Solar System, will be superseded in the remote depths of intergalactic space. The dark halos that are inferred to surround galaxies would then turn out to be a mere illusion. The overwhelming majority of cosmologists, however, are convinced by the reality of the dark matter. No longer is the epithet "missing" matter appropriate: the gravitational probes developed by taking spectra of gas clouds and dwarf galaxies in remote circumgalactic orbits confirm the inexorable tug of the dark matter. An impressive array of experiments has been developed in recent years to search for this pervasive but elusive component of the Universe.

These two books are devoted to describing the theory of dark matter. *The Fifth Essence* is by Lawrence M. Krauss, a pioneer of the evolving discipline of particle astrophysics. This new field is at the interface of particle physics and astronomy, and has provided a major industry for astrophysicists. The search for dark matter is a prime example of what drives particle astrophysics. Hints abound from cosmologists that something new is needed to explain their observations. Particle physicists, impatiently awaiting the construction of ever larger and more expensive particle accelerators, eagerly seize the opportunity provided by the astrophysical laboratory for testing their newest theories. And with good reason: energies achieved routinely in the very early Universe exceed by many orders of magnitude those attainable in the more grandiose accelerators now being planned. Dark matter may plausibly consist of some exotic weakly interacting particle, thereby naturally accounting for its domination of the outermost, low-density environs of galaxies, whereas the strongly interacting stuff of which stars are made dissipates freely and is concentrated in the inner luminous cores of galaxies.

Naturalness does not by any means stop here if you are a particle theorist.

Krauss embarks on a meandering journey through the various symmetries of the fundamental interactions. The going is not easy, but I found the description of the axion, named after a laundry detergent for reasons that he does not explain, a brave and lucid attempt to clarify some of the more esoteric aspects of elementary physics. Krauss does have a tendency to launder the language ("that's piddling", "it's a snap"), and tells us on three separate occasions (all neatly indexed) that Alan Guth is a young particle physicist. The astronomical aspects of cosmology are well covered: we learn about the microwave background and the puzzles of large-scale structure. Some mysteries remain: for example, the reader is left wondering why two Nobel laureates thought pigeon droppings might be a source of omnidirectional radio noise. Occasional errors creep in: the excess of high-frequency energy in the microwave background was reported by a rocket-flown, not a satellite-based, experiment. But the highlights include enthralling descriptions of the direct searches for dark matter being mounted deep underground by several groups around the world.

Invisible Matter and the Fate of the Universe, by Barry Parker, provides a very different approach to dark matter. Parker makes no pretence of explaining the arcane mysteries of particle physics, but provides capsule summaries of practically all important astronomical discoveries even remotely related to dark matter. His style emphasizes personalities, to the extent of incorporating informal quotations from many of the featured astrophysicists, together with their photographs. His journalistic approach provides the flavour of current research while allowing him to avoid getting bogged down in the technical background. Gaps are inevitable, but Parker succeeds in making the latest ideas accessible.

The author's principal weakness is that he is uncritical. He quotes Arp on discordant redshifts, and Tifft on their quantization, with the same emphasis that he places on less controversial theories. "We don't know" summarizes his reaction. Fair enough, but the naive reader will stumble inadvertently into a minefield of controversy without more guidance.

Some cosmologists get short shrift, Fred Hoyle in particular. But it was Hoyle who first conjectured that the angular momentum of galaxies may arise from tidal torques between protogalaxies, and it was Hoyle who, with Roger Tayler, resurrected Gamow's idea that primordial nucleosynthesis formed helium in the hot Big Bang. I was left puzzled by some remarks. Why should dark matter in the form of dwarf stars (red, white, brown and black) make galaxy halos any "stickier" than alternative forms of dark matter?

The discussion of black holes is confusing. John Mitchell and Pierre Laplace noticed in the eighteenth century that a star equal in mass to the Sun but one-hundred-thousandth of its radius would possess a gravitational field so strong that light could not leave it. Very large, but still finite, pressure is attained by gravitational contraction of sphere to its Schwarzschild radius. No infinity there, contrary to what Parker states. He seems to be unaware of the gallium experiments now being mounted to search for neutrinos from the Sun. And what is the meaning of the claim that Dirac's monopole has a tail, or a string, attached? In summary, despite the breathless style, the book is fun to read.

These two volumes make a good combination. The concept of a Universe dominated by dark matter is difficult to swallow, but the evidence is conclusive. Try *Invisible Matter* for an aperitif, and *The Fifth Essence* to provide the entrée. □

Joseph Silk is in the Department of Astronomy, University of California, Berkeley, California 94720, USA.

Protecting all interests?

Barry Cross

Animal Patents: The Legal, Economic and Social Issues. Edited by William H. Lesser. *Stockton, New York*: 1989. Pp. 369. £55, \$100.

FEW issues are more fiercely argued today than those surrounding the creation of new varieties of animal life in the test tube. Shortly before Christmas, a much publicised debate on BBC television in Britain ended in a two-to-one vote in favour of a bill of rights for animals. The notion of patenting animals is anathema to many, and the Patents Act of 1977 precludes this practice for animals bred in the normal way, although whether genetically engineered animals can be patented has not been tested in the British courts. The European Patent Office, however, seems to be moving in the direction of recognizing the patentability of living matter. If biotechnology companies, which invest heavily in new developments in animal breeding, are not given patent protection they will be unable to compete in international markets. So we are witnessing the familiar conflict between economic advantage and moral objection, and it is quite possible that the European Parliament will take a different view from that of the European Patent Office.

Animal Patents is not for the faint-hearted. Half the pages are devoted to thirteen essays on legal, technical,

economic and user issues and the other half to a compendium of appendices comprising legislative, judicial and patent documents. Nevertheless, the book offers a fascinating insight into the constitutional procedures by which such contentious matters are handled in the United States, a country that is far ahead of Europe. Despite the highly vocal animal-rights movement — with such notable advocates as Jeremy Rifkin — the US government has moved fast to protect the interests of inventors, farmers and other consumers.

Baruch Brody contributes a useful essay on the moral and ethical objections to patenting animals, reaching the conclusion that the opponents "must give us some reason to support (and accept the radical implications of) their rethinking the entire relation between humanity and the natural order". Robert Foote examines the implications of the new technology for animal science, and proposes that cryogenic embryo banks can solve the legal requirement to deposit examples of the patented product. Another distinguished animal scientist, William Hansel, discusses the likely impact of transgenic farm animals, and the economic consequences are further explored by Robert Milligan and William Lesser, who particularly welcome the major funding by the private sector of biotechnology centres in US universities and conclude that "royalties are necessary to encourage the needed investment in animal biotechnology research".

Pages 161–171 represent the heart of the book, for they contain US Patent No. 4736866, Leder *et al.*, dated 12 April 1988, entitled "Transgenic Non-Human Animals". Anyone interested in molecular biology or genetic manipulation must be stirred by the historic precedent created by "oncomouse", the Harvard mouse containing a recombinant activated oncogene sequence for use in cancer research. Equally absorbing is the account of the second session of the hundredth US Congress on 26 August, when the gentleman from Wisconsin, Mr Kastenmeier, presented the Report of the Committee of the Judiciary on the Transgenic Animal Patent Reform Act. This report embodies a formidable review of the relevant science and technology amid a welter of submissions from civil-rights groups and patent lawyers. A letter from the White House urges acceptance of animal patents in the national economic interest because "Ten countries now permit animal patents, including Japan and Canada, while another 53 have not prohibited the granting of patents." Leaning on legislators is a well-recognized Top People sport in Britain too. □

Sir Barry Cross is Secretary of the Zoological Society of London and was Director of the AFRC Institute of Animal Physiology and Genetics Research, Babraham, Cambridge CB2 4AT, UK.



Bright lights — auroras are the result of ionization of earth's atmospheric gases, caused by solar electrons channelled there by the earth's magnetic field, at altitudes of 100 to 200 km above the magnetic poles. The picture here is reproduced from *The Scientific Companion* by C. Emiliani. Published by Wiley, 1988, and now available in paperback, price \$14.95, £9.95.

Thyroid gap

Reg Hall

The Story of Iodine Deficiency: An International Challenge in Nutrition. By Basil S. Hetzel. Oxford University Press: 1989. Pp. 236. Hbk £18.50, \$35; pbk £6.95.

BASIL Hetzel is one of the pioneers in the field of iodine deficiency. He has studied the problem as a professor of medicine, as a public health administrator and as an epidemiologist in the field. In this concise book he highlights the present situation on iodine deficiency, which even today is a hazard for nearly a billion people. Hetzel has collaborated with many eminent workers in the field, notably John Stanbury, V. Ramalingaswami and John Dunn, and with them has set up the International Council for Control of Iodine Deficiency Disorders, a body which aims to close the gap between current knowledge in the field and its application.

Part I of Hetzel's book deals with the understanding of iodine deficiency and introduces the author's concept of the iodine deficiency disorders, comprising goitre, still births and miscarriages, neonatal and juvenile thyroid deficiency, dwarfism, mental defects, deaf mutism and spastic weakness and paralysis, as well as lesser degrees of loss of physical and mental function. All these defects result from lack of thyroid hormone, of which iodine is an essential constituent, and all of them can be prevented by adequate iodine intake.

In part II, Hetzel deals with the international control of iodine deficiency disorders by iodine supplementation. He highlights the logistical problems of achieving adequate iodine intake in many parts of the world. In the West, iodine deficiency disorders have been prevented by methods such as the iodine supplementation of salt, or sometimes by chance, for example in the United Kingdom where cattle food supplements in winter provide a variably large iodine intake in milk and dairy foods. But deficiency still exists in parts of Europe, such as southern West Germany, where compulsory iodine supplementation is forbidden by law. In the developing world, progress remains slow; large populations in China, India, Indonesia and Africa remain iodine deficient.

In part III the author summarizes the present status of iodine deficiency disorders and their control in Europe, Africa, Latin America and Asia. He concludes that the chances of significant progress for correction of these disorders will depend on the "motivation of national governments", the financial resources and the availability of trained manpower.

Altogether this is a splendid book by an acknowledged authority in the field. It will appeal to a wide audience of physicians, nutritionists, field workers and epidemiologists as a concise, simple introduction to the problem of iodine deficiency. □

Reg Hall was Professor of Medicine at the University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, UK.

Lexicon from the laboratory

Gareth Jones

The Dictionary of Cell Biology. Edited by J. M. Lackie and J. A. T. Dow. *Academic*: 1989. Pp.262. Hbk £19, \$34.95; pbk £9.95.

DO WE need a dictionary for cell biologists? Virtually all the teaching staff in the cell biology department of the University of Glasgow seem to feel that we do, not so much for our own use but for those whom we teach. The editors state that the aim of this book is to provide students and other "learners" with a quick and painless introduction to the common jargon of cell biology, providing very brief descriptions of a vast range of words and abbreviations compiled from the indexes of the chief textbooks and the key-word libraries of prestigious journals.

Although the editors and authors can be praised for the excellent idea, the execution must have been fraught with problems. It takes only a casual glance through the book to see that conflicts must have arisen constantly between the demands for brevity and for clarification. Whereas the reader of a standard dictionary might be satisfied by the definition of "dermis" as "of the skin", a student would need more, but how much more — is "mesodermally-derived connective tissue underlying the epithelium of the skin" enough? Two methods have been used here to address this problem. There is some cross-referencing, which allows the persistent reader to follow through a definition. There is also the much more useful ploy of providing tables, such as for the classes of intermediate filaments, which provide more of the data really needed by a student cell biologist.

Whatever other attributes one could wish for this book, the very nature of a dictionary demands that the definitions are accurate. Only the very best of us can honestly say that they are not unduly influenced by what they read versus what they are told in lectures, for instance. Unfortunately, I found rather too many errors for my liking, most starkly demonstrated in a table of the classification of integrin subtypes, where the alpha- and beta-chain designations are transposed.

Despite these criticisms, I think the authors and editors have made a good start in their praiseworthy attempt to provide easy access to words in common usage. I suspect any future editions will see longer entries, with expanded use of tables, and closer attention to detail. □

Gareth Jones is in the Anatomy Department, Division of Biomedical Sciences, King's College, Strand, London WC2R 2LS, UK.

Following the rise of the Sun

Koscak Maruyama

The Formation of Science in Japan: Building a Research Tradition. By James R. Bartholomew. *Yale University Press*: 1989. Pp.371. \$36, £25.

ALTHOUGH Japan's GNP is one of the highest in the West, what is the quality of its science? Few Japanese scientists are prepared to speak out on this issue, an exception being Nobel physicist Leo Esaki, who openly states his belief that Japan has failed to shake free of its reputation for imitative, rather than original, scientific research. Japan's technological strength does not emerge from a broad base of original ideas, and the country's contribution to learning is minimal, in Esaki's view, because Japanese society is group-orientated. On the face of it, these statements seem correct — there have been only 5 Japanese Nobel laureates in science out of a total of 395 winners.

But are the characteristics of efficient imitation and group solidarity alone, as suggested by Esaki, sufficient to explain Japan's economic success? Most Japanese scientists would hesitate to become involved in this controversial question, but the author of this book, who is from the United States, has attempted to address it. Bartholomew explains the unique background to the growth of modern scientific research in Japan, emphasizing the pockets of genuine originality that existed despite the great handicap to growth and development caused by the country's isolation from the West between 1639 and 1853. Bartholomew points out that Esaki's pessimism is not entirely justified — Esaki's own discovery of the tunnel diode effect was made in Japan, although at a company institute rather than a university.

During the Tokugawa period in Japan (1600–1867), individualism was inhibited by the feudalistic nature of society and a strict caste system. Physicians trained in the Chinese tradition helped to introduce Western medicine, and Dutch medical scientists at Nagasaki, who were Japan's only regular contact with Europe, provided some exposure to Western ideas — the Dutch edition of J. A. Kalmus's *Anatomische Tabellen* (1772) was translated into Japanese, for example. Some scientific progress was made. Seishu Hanaoka excised a breast tumour under general anaesthesia in 1805, 40 years earlier than the famous ether anaesthesia performed at Massachusetts General Hospital. Takakazu Seki devised a limited form of calculus and Tobei Kunitomo observed sunspots in 1835, publishing a

drawing of the surface of the Moon obtained by the use of a hand-held telescope. But on the whole, science did not properly develop during the period of isolation, one reason being the Confucian morality that students should not under any circumstances oppose their teachers.

When *Meiji* (restoration/modernization) began in 1868, most Japanese scientists were capable only of transcribing foreign books, although there were a few people who could do original research, particularly in the field of medicine. The new government sent many young scientists to the West, mainly Germany, the United States, Great Britain and France. As an example, Bartholomew refers to Shibasaburo Kitasato (rather excessively, on more than 60 occasions!) who learned bacteriology from a Dutch navy doctor at a local medical school and, after graduation from Tokyo University, went to Germany to study under Robert Koch in the late nineteenth century. Kitasato isolated tetanus bacteria, produced tetanus and diphtheria vaccines, and established the concept of immunity in collaboration with E. von Behring. Bartholomew argues that Kitasato should have shared the first Nobel prize with von Behring. After his return to Japan, Kitasato established a private institute for infectious diseases in Tokyo, which later became a national institute under the Ministry of Home Affairs. Kitasato discovered that *Pasteurella pestis* was the cause of the plague in 1894. He gave up the directorship of the institute when infighting among its academics forced its transfer to the Ministry of Education. Kitasato created another institute, the Kitasato Institute in Tokyo, with various eminent collaborators. Bartholomew's description of Japanese academic and bureaucratic factionalism is vivid and exciting.

Another of Bartholomew's examples is Katsusaburo Yamagiwa (1863–1930), Professor of Pathology at Tokyo University. In 1914, Yamagiwa was the first to succeed in producing cancer in a rabbit, which he did by repeatedly applying coal tar to its ear. The active substance producing the effect, 3,4-benzpyrene, was identified by E. L. Kennaway in 1932. Bartholomew contends that the Nobel prize committee made a great mistake in omitting to nominate Yamagiwa for the 1926 prize, which was given to J. Fiebigler for his erroneous theory of the cause of cancer.

It was not easy for scientific institutions to be created in Japan. Tokyo University was founded in 1877, and Bartholomew gives details of the founding of Kyoto, Kyushu, Hokkaido and Tohoku universities. The founding of the Research Institute for Physics and Chemistry in 1917 is noteworthy because this institute, half supported by the state, produced

first-class scientific contributions in an atmosphere of academic freedom. Nobel laureates Hideki Yukawa and San'ichiro Tomonaga worked there, for example. Nevertheless, expansion of scientific institutions was restricted, and Bartholomew's account shows clearly the origins of today's political bureaucracy.

Bartholomew covers the formation of science in Japan up to the 1920s. His is a crisp, readable account, based on numerous government documents and other sources written in Japanese. No study as thorough as this one exists, even in Japanese, and it is full of little-known facts — for example, I did not know that the present Ministry of Education's research grant began as early as 1918.

In 1939, television broadcasts were demonstrated for the first time at the World Fair in New York. It took only 14 years for television to reach Tokyo, and today every US resident enjoys watching Japanese-made television sets. Bartholomew provides an explanation for the underlying basis of Japan's technological successes up until the 1920s: efficient imitation of Western science — but with some original developments — after a long period of isolation. He now has another task in analysing the post-war boom in Japanese science and technology. □

Kosack Maruyama is in the Department of Biology, Faculty of Science, Chiba University, Yayoi-cho, Chiba 260, Japan.

Clever chaps

John Currey

On Biomineralization. By Heinz A. Lowenstam and Stephen Weiner. *Oxford University Press: 1989. Pp. 324. £40, \$57.*

Biomineralization. By Kenneth Simkiss and Karl M. Wilbur. *Academic: 1989. £50, \$69.95.*

Biomineralization: Chemical and Biochemical Perspectives. Edited by S. Mann, J. Webb and R. J. Williams. *VCH: 1989. Pp. 541. DM 274, \$98.*

ALL the main groups of organisms contain members that make use of minerals in their bodies. Most obviously, a mineral is used to produce skeletons for protection and support, but minerals are put to many other uses — as gravity and inertia sensors, as sensors of the Earth's magnetic field, as optical systems and as stores of the ions of the mineral. Materials scientists faced with the task of making skeletal materials within the constraints that living organisms endure would probably resign. Among these constraints are that the materials must be available locally, that the processing must take place almost at equilibrium at all times, in the temperature range 0 to 40 °C, without any large energy inputs and that, as organisms grow, the skeleton must be functional at all stages in the life history.

Organisms do have some advantages, however. Often the rate of formation of the skeleton is not critical. Also, organisms can control the ionic constitution of their body fluids, and of fluids just outside their bodies, with extraordinary precision, and the composition can be varied by orders of magnitude over very small distances. The tonoplast membrane of plant cells separates compartments differing in calcium-ion concentration by a factor of 10^5 over a distance of 10 nanometres. Such precision enables organisms to produce structures of great delicacy or robustness

as required. The study of these developmental processes is coming into its own, and the rather remarkable appearance of three substantial books with virtually the same title, within a few months of each other, is an indication of this.

Two of the books, by Lowenstam and Weiner (Lowenstam), and Simkiss and Wilbur (Simkiss), are very similar in their approach. They deal with general issues such as the control of mineralization (Simkiss), biogeochemical cycles (Simkiss), environmental influences on biomineralization (Lowenstam) and the evolution of biomineralization (Lowenstam), but their main focus is taxonomic, with discussion of the kinds of biomineralization processes found in the major groups, and of the anatomy and microanatomy of the structures produced. Both books are well illustrated, particularly Simkiss. Lowenstam is about 50 per cent longer, and goes more deeply into a number of subjects. Simkiss is written at a slightly lower technical level, containing more deft turns of phrase and interesting asides. Both books are remarkably readable considering their complex subject matter. The ability of organisms to mould the form of minerals coming out of solution is at times almost literally incredible. I found myself turning the pages with rather naive excitement, wondering "whatever next?"

The profusely illustrated book edited by Mann, Webb and Williams covers much of the same ground but at considerably greater length. Each of the fifteen chapters tends to be focused on a particular, often rather restricted topic. As a result there is no real attempt at a comprehensive coverage. The discussion of vertebrate hard tissue, for instance, is limited almost entirely to 29 pages by Viess on the organic components of teeth. There is virtually no mention of bone or eggshells, and otoconia are mentioned in passing elsewhere in the book. Simkiss gives a few pages to each of these last topics, but does not mention dental tissues. Lowenstam gives the full treatment — more than 50

pages — to chordate hard tissues, but barely mentions eggshells.

This eclectic approach is not taken to extremes in Mann *et al.*, in which mineralization in most groups is discussed. Some chapters are a delight; those by Mann and Frankel on magnetite in microorganisms, by Addadi and Weiner on organic/inorganic relations in mineralization, and by Mann on "Crystallochemical Strategies in Biomineralization", are particularly interesting, but there will be certainly many others of value to anyone interested in biomineralization. In general, of course, the extended coverage gives a more satisfying account of the chosen topics than is found in the other two books. Mann *et al.* contains several chapters on analytical techniques that are becoming increasingly important, such as nuclear magnetic resonance, infrared, ultraviolet, and Mössbauer spectroscopies, high-resolution transmission electron microscopy, and proton beam analysis.

It is always a temptation to criticize books for not doing what they do not attempt; both Simkiss and Mann *et al.* emphasize their chemical, biochemical and cell-biological orientation in their subtitles. But it is disconcerting that these books reflect so little consideration of the function of the finished products. This lack is particularly obvious in the chapters devoted to skeletons. Foraminiferans and coccolithophorids have extremely complex skeletons, whose shapes are virtually fixed within species but vary considerably between species. For example, I would like to know whether this matters to the organism; does the precise shape of its skeleton have an adaptive value that would be less if the skeleton were different? Again, the organic matrix is repeatedly shown in these books to have a crucial role in the control of mineralization. Yet we also know that microanatomy produced by the presence of the organic matrix, and the organic matrix itself, have a profound effect on the mechanical properties of the resulting skeleton. Except for a short section in the chapter by Birchall in Mann *et al.*, dealing with the insights to be gained by technologists from biomineralization, this dual role of the organic constituents of mineralized skeletons is barely mentioned.

Publication of these three books leaves graduate students starting work on biomineralization in an enviable position. There are now two excellent accounts providing an overview of the subject; they should certainly buy one. And the third gives insight into the state of research in many parts of the field, research that is beginning to produce exciting answers to difficult questions. The subject is growing up. □

John Currey is Professor of Biology, University of York, York YO1 5DD, UK.

Influences of cloud photochemical processes on tropospheric ozone

J. Lelieveld & P. J. Crutzen

Max Planck Institute for Chemistry, Division of Atmospheric Chemistry, PO Box 3060, D-6500 Mainz, FRG

Global models of atmospheric chemistry typically neglect the chemical effects of clouds. Although clouds occupy only about 15% of the volume of the lower troposphere, and the volume fraction of liquid water in clouds is only 10^{-7} to 10^{-6} , aqueous-phase chemistry is shown to decrease significantly ozone concentrations in the troposphere. Hydroxyl, formaldehyde and nitrogen oxide concentrations are decreased as well, thereby reducing the oxidation and cleansing efficiency of the troposphere and influencing the atmospheric budgets of many trace gases.

CLOUDS are of great importance in tropospheric chemistry. Upward transport of surface-emitted trace gases mostly takes place in clouds. Precipitating clouds return particulate matter and water-soluble gases to the Earth's surface. Further, clouds scatter and reflect photochemically active solar ultraviolet radiation. Finally, important chemical reactions occur in clouds. Regarding the latter, there has been much emphasis on the aqueous-phase oxidation of sulphur dioxide to sulphuric acid, the major constituent of acid rain¹. But studies that treat the overall role of clouds on the photochemistry of background air are not available, although the basis for such studies was laid down in refs 2–4. In this exploratory study we use a cloud-photochemistry model to investigate the potential effects of clouds on ozone production and destruction rates. To illustrate global-scale effects, we focus on several altitudes in the tropics and mid-latitudes during summer and winter. Because we concentrate on the chemical effects of clouds, we simplify a number of cloud physical and radiative properties. The results indicate that in NO_x -rich environments ($\text{NO}_x = \text{NO} + \text{NO}_2$) clouds reduce net O_3 production. Moreover, in NO_x -poor areas net O_3 destruction is enhanced. Consequently, photochemical models that do not consider the role of clouds will considerably overestimate ozone concentrations. Clouds also reduce the oxidation efficiency of the troposphere, as determined by OH and H_2O_2 . We further show, that the presence of clouds causes reductions of the concentrations of NO_x and formaldehyde (CH_2O), and of the rates of CO and H_2 production from methane oxidation.

Gas-phase chemistry

The photochemistry of the background troposphere is to a large extent controlled by reactions of O_3 , H_2O , CH_4 , CO and NO_x that are initiated by ultraviolet radiation (the key reactions are listed in Table 1). These reactions introduce hydroxyl radicals (OH), as well as H_2O_2 , which regulate the oxidation efficiency of the troposphere in the gaseous and aqueous phases. The concentrations of these gases are largely determined by reactions in the oxidation cycles of CO and CH_4 , which also have a regulatory role in the ozone budget of the troposphere⁵.

Whenever NO volume mixing ratios exceed those of O_3 by more than the ratio of the rate coefficients between reactions G16 and G22 (or G28), for example, 2.5×10^{-4} at 298 K, ozone production by reaction G22 (or G28), followed by reactions G3 and G13, becomes more important than ozone destruction by

reaction G16. At the Earth's surface this corresponds to a volume mixing ratio of $\text{NO} \geq 5$ p.p.t.v. (5 parts per 10^{12} by volume, approximately equivalent to 15–20 p.p.t.v. NO_x). Because of ozone loss by reactions G1 + G14, however, net ozone production takes place only at larger NO_x volume mixing ratios, depending on latitude and season. Especially in warm humid air, reaction G14 may be so important that net production of O_3 requires concentrations of NO_x that are a few times larger. Over the Northern Hemisphere, where NO_x emissions are considerably enhanced by anthropogenic activities, photochemical O_3 production is a common phenomenon. In more pristine ocean environments and in much of the Southern Hemisphere O_3 destruction tends to dominate, so that in these regions the presence of O_3 may be largely the result of transport from the stratosphere.

Formaldehyde is an important precursor of HO_x ($\text{OH} + \text{HO}_2$), CO and H_2 through photodissociation reaction G5, reactions G5 + G6 + G31 and reaction G6, respectively. The amount of CO that is produced through CH_4 oxidation is about one-quarter of the global atmospheric CO input⁶. The remainder originates from the oxidation of hydrocarbons other than CH_4 , fossil-fuel combustion and biomass burning—the latter mainly in the tropics. In marine environments of the Southern Hemisphere, the influence of continental emissions is relatively small, so that in these regions photochemical production from CH_4 oxidation may be the largest source of CO. The photolysis of CH_2O (G6) accounts for about one-quarter of the total H_2 input in the troposphere⁷.

Chemical effects of clouds

After entering a cloud, formaldehyde partly dissolves in the droplets where it almost immediately forms $\text{CH}_2(\text{OH})_2$. The latter reacts rapidly with aqueous-phase OH radicals (reaction A3), which is a source of formic acid (HCOOH) (ref. 8). HCOOH in turn is also quite efficiently oxidized by OH in the droplets by reactions A4 and A5, so that the global average contribution to HCOOH concentrations through this mechanism is limited. When we consider only reaction A3 as a source, calculated HCOOH volume mixing ratios are only a few tens of p.p.t.v., whereas measurements indicate much higher values⁹. Reactions A3–5 all consume OH, but produce HO_2 , so that there is neither loss nor gain of HO_x radicals. This implies that dissolution of CH_2O in clouds prevents net production of HO_x by reaction G5. The reaction between H_2O_2 and OH (A9) also produces HO_2 . Because two HO_2 molecules are required to form one H_2O_2 molecule (A7, G21), reaction A9 is a sink for HO_x . The reaction sequence A14–17, initiated by the reaction between dissolved CO_2 (HCO_3^-) and OH, does not exert a significant net effect on the aqueous-phase HO_x budget. These reactions therefore hardly affect the chemistry in air masses containing typical background CH_2O and H_2O_2 concentrations.

Another source of HO_2 in the droplets is scavenging from the gas phase, which is extremely efficient. A large fraction of the dissolved HO_2 (dependent on the pH) dissociates into H^+ and O_2^- (E2). The superoxide ion can react rapidly with ozone (A6). Despite the relatively low solubility of O_3 , reaction A6 is the dominant source of OH in the aqueous phase. The OH flux from this reaction can be 5–50 times larger than scavenging of OH from the gas phase (at 285–268 K, respectively). Hence, OH is recycled through aqueous-phase chemistry and most of the

free radicals formed react with $\text{CH}_2(\text{OH})_2$, H_2O_2 or HCOO^- again. Because of the rapidity of aqueous-phase reaction A3, the breakdown of CH_2O can be over ten times faster in clouds than in the cloudless troposphere⁴. Therefore, the presence of clouds leads to lower gas-phase CH_2O concentrations and less production of HO_x by reaction G5. Unlike many reactions, there is no aqueous-phase counterpart of reaction G5. Furthermore, breakdown of CH_2O in the aqueous phase produces CO_2 and H_2O , not CO and H_2 .

The efficient scavenging of HO_2 from the gas phase affects the O_3 , H_2O_2 and HO_x chemistry in the interstitial air. As previously discussed, an important reaction under cloudless conditions is that between HO_2 and NO , which leads to the

formation of NO_2 and subsequently of O_3 . In clouds, however, this reaction is suppressed. Whereas HO_2 is largely scavenged by the droplets, NO , which dissolves very poorly, is not. Hence, NO and HO_2 become separated and ozone production in clouds through reactions $\text{G22} + \text{G3} + \text{G13}$ is strongly reduced. The same argument applies to the reaction (G28) between CH_3O_2 and NO . O_3 formation through G28 is also reduced, although to a lesser extent than reaction G22, because CH_3O_2 is not so strongly depleted as HO_2 . In addition, O_3 is destroyed by O_2^- in clouds through reaction A6. Hence, in clouds HO_2 (through O_2^-) always causes net O_3 destruction, whereas in a cloudless atmosphere with sufficient NO_x , O_3 is produced.

The O_2^- required for reaction A6 is provided by scavenging of HO_2 from the gas phase, and by the reactions of OH with $\text{CH}_2(\text{OH})_2$, HCOOH (A3-5) and H_2O_2 (A9), followed by equilibrium E2. In cold environments (268 K, for example), when a large fraction of these gases is dissolved, $\text{CH}_2(\text{OH})_2$, HCOOH and H_2O_2 are about five times more important as aqueous-phase HO_2 sources than scavenging of HO_2 from the gas phase. In relatively warm air, (285 K, for example) HO_2 scavenging from the interstitial gas phase is approximately as important as HO_2

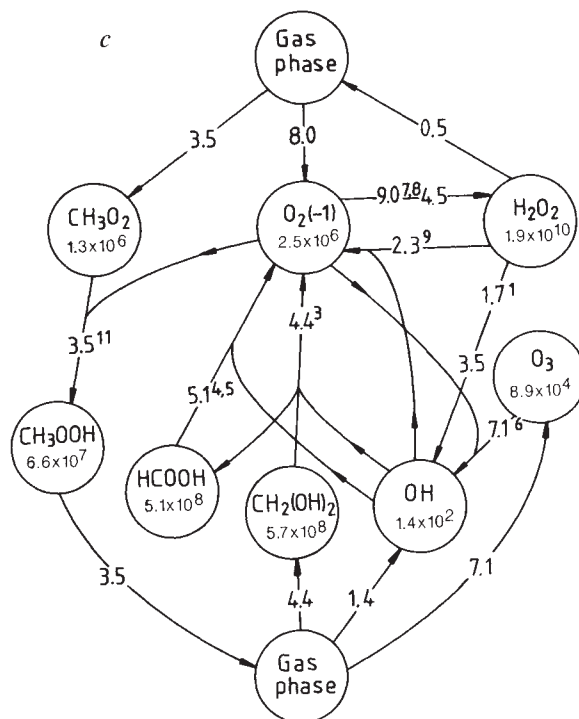
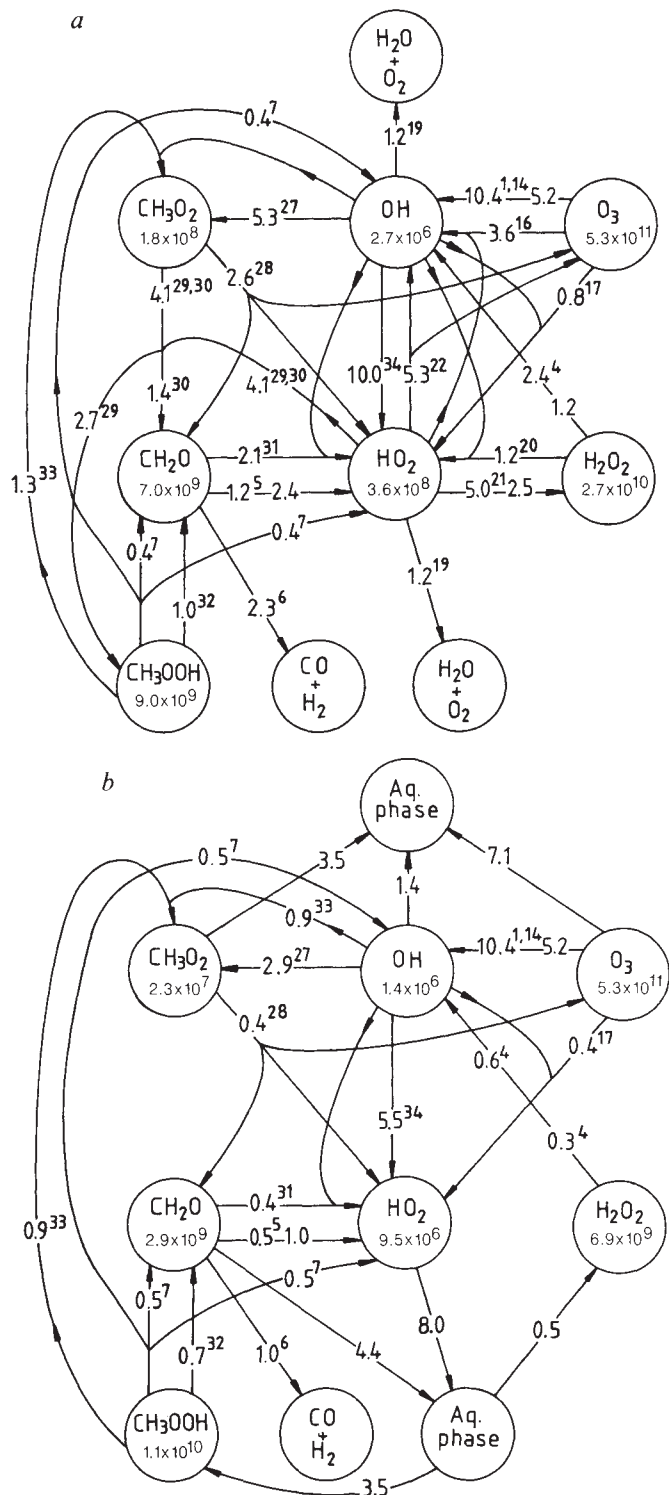


FIG. 1 Calculated HO_x fluxes for conditions at the Equator at 3 km altitude. Concentrations within the circles are in molecule cm^{-3} . Fluxes (represented by the lines) are in units of 10^5 molecule $\text{cm}^{-3} \text{s}^{-1}$. The superscripts denote the gas-phase reactions or the aqueous-phase reactions in Table 1. Some lines have two numbers, because the associated reactions produce or consume 2HO_x against one molecule of the other reactant. Only fluxes exceeding 3.0×10^4 molecule $\text{cm}^{-3} \text{s}^{-1}$ are depicted. *a* pertains to a case without clouds, *b* and *c* to the corresponding case with cloud, 1.5 h after cloud formation. *b* shows the interstitial gas-phase fluxes, *c* the aqueous-phase fluxes and concentrations. The rate of reaction A3 is limited by aqueous-phase diffusion of OH . Aqueous-phase concentrations in *c* are given as gas-phase equivalent concentrations in molecule cm^{-3} and fluxes accordingly, to facilitate comparison between both phases. $\text{O}_2(-1)$ in *c* is the sum of HO_2 (aq) and O_2^- . HCOOH in *c* is the sum of HCOOH (aq) and HCOO^- . To convert to aqueous-phase concentrations in mol l^{-1} , all concentrations within the circles in *c* should be multiplied with 4.17×10^{-15} . Reactions A14-17, although included in the model, are almost neutral with respect to HO_x . These reactions are, therefore, not included in *c*. *b* is the same as *a*, with the exception that the aqueous phase is added. Note that many chemical fluxes involving HO_2 destruction have disappeared, because they dropped to a level below 3.0×10^4 molecule $\text{cm}^{-3} \text{s}^{-1}$. We note that CH_2O in particular, is destroyed by photochemical reactions.

TABLE 1 Gas-phase, gas-liquid equilibrium and aqueous-phase reactions

Gas-phase reactions			Rate constant*	Ref.	
G1	$O_3 + h\nu \rightarrow O(^1D) + O_2$		1.6×10^{-5}	27	
G2	$O_3 + h\nu \rightarrow O + O_2$		3.6×10^{-4}	27	
G3	$NO_2 + h\nu \rightarrow NO + O$		5.6×10^{-3}	27	
G4	$H_2O_2 + h\nu \rightarrow 2OH$		4.6×10^{-6}	27	
G5	$CH_2O + h\nu + 2O_2 \rightarrow 2HO_2 + CO$		1.7×10^{-5}	27	
G6	$CH_2O + h\nu \rightarrow H_2 + CO$		3.3×10^{-5}	27	
G7	$CH_3OOH + h\nu + O_2 \rightarrow CH_2O + HO_2 + OH$		4.6×10^{-6}	27	
G8	$NO_3 + h\nu \rightarrow NO + O_2$		1.4×10^{-2}	27	
G9	$NO_3 + h\nu + O_2 \rightarrow NO_2 + O_3$		1.2×10^{-1}	27	
G10	$HNO_3 + h\nu \rightarrow NO_2 + OH$		3.2×10^{-7}	27	
G11	$N_2O_5 + h\nu \rightarrow NO_2 + NO_3$		2.7×10^{-5}	27	
G12	$O(^1D) + M \rightarrow O + M$		$2.0 \times 10^{-11} \exp(100/T)$	27	
G13	$O + O_2(+M) \rightarrow O_3(+M)$		1.5×10^{-15}	27	
G14	$O(^1D) + H_2O \rightarrow 2OH$		2.2×10^{-10}	27	
G15	$O_3 + NO \rightarrow NO_2 + O_2$		$2.0 \times 10^{-12} \exp(-1,400/T)$	27	
G16	$O_3 + HO_2 \rightarrow OH + 2O_2$		$1.1 \times 10^{-14} \exp(-500/T)$	27	
G17	$O_3 + OH \rightarrow HO_2 + O_2$		$1.6 \times 10^{-12} \exp(-940/T)$	27	
G18	$NO_2 + OH(+M) \rightarrow HNO_3(+M)$		1.2×10^{-11}	27	
G19	$HO_2 + OH \rightarrow H_2O + O_2$		$4.6 \times 10^{-11} \exp(230/T)$	27	
G20	$H_2O_2 + OH \rightarrow HO_2 + H_2O$		$3.3 \times 10^{-12} \exp(-200/T)$	27	
G21	$HO_2 + HO_2 \rightarrow H_2O_2 + O_2$		$2.3 \times 10^{-13} \exp(600/T)$	27	
G22	$HO_2 + NO \rightarrow NO_2 + OH$		$3.7 \times 10^{-12} \exp(240/T)$	27	
G23	$NO_2 + O_3 \rightarrow NO_3 + O_2$		$1.4 \times 10^{-13} \exp(-2,500/T)$	27	
G24	$NO + NO_3 \rightarrow 2NO_2$		$1.7 \times 10^{-11} \exp(150/T)$	27	
G25	$NO_2 + NO_3(+M) \rightarrow N_2O_5(+M)$		$8.1 \times 10^{-11} (T/300)^{-4.1}$	28	
G26	$N_2O_5(+M) \rightarrow NO_2 + NO_3(+M)$		$4.6 \times 10^{16} (T/300)^{-4.4} \exp(-11,080/T)$	28	
G27	$CH_4 + OH + O_2(+M) \rightarrow CH_3O_2 + H_2O(+M)$		$2.3 \times 10^{-12} \exp(-1,700/T)$	27	
G28	$CH_3O_2 + NO + O_2 \rightarrow CH_2O + HO_2 + NO_2$		$4.2 \times 10^{-12} \exp(180/T)$	27	
G29	$CH_3O_2 \rightarrow CH_3OOH + O_2$		4.0×10^{-12}	29	
G30	$CH_3O_2 + HO_2 \rightarrow CH_2O + H_2O + O_2$		2.0×10^{-12}	29	
G31	$CH_2O + OH + O_2 \rightarrow HO_2 + H_2O + CO$		1.1×10^{-11}	28	
G32	$CH_3OOH + OH \rightarrow CH_2O + OH + H_2O$		4.4×10^{-12}	30	
G33	$CH_3OOH + OH \rightarrow CH_3O_2 + H_2O$		5.6×10^{-12}	30	
G34	$CO + OH + O_2 \rightarrow CO_2 + HO_2$		2.4×10^{-13}	27	
G35	$CH_3O_2 + CH_3O_2 + O_2 \rightarrow 2CH_2O + 2HO_2$		$1.9 \times 10^{-13} \exp(220/T)$	27	
Gas-aqueous and aqueous-phase equilibria			$K_{298}^\dagger$	$-\Delta H/R$	Ref.
E1	$HO_2(\text{gas}) \rightleftharpoons HO_2(\text{aq})$		2.0×10^3	6,600	31
E2	$HO_2(\text{aq}) \rightleftharpoons O_2^- + H^+$		3.5×10^{-5}		32
E3	$H_2O_2(\text{gas}) \rightleftharpoons H_2O_2(\text{aq})$		7.4×10^4	6,615	33
E4	$O_3(\text{gas}) \rightleftharpoons O_3(\text{aq})$		1.1×10^{-2}	2,300	34
E5	$CH_2O(\text{gas}) \rightleftharpoons CH_2(OH)_2$		6.3×10^3	6,425	8
E6	$HCOOH(\text{gas}) \rightleftharpoons HCOOH(\text{aq})$		3.7×10^3	5,700	35
E7	$HCOOH(\text{aq}) \rightleftharpoons HCOO^- + H^+$		1.8×10^{-4}	-1,510	35
E8	$CH_3OOH(\text{gas}) \rightleftharpoons CH_3OOH(\text{aq})$		2.2×10^2	5,653	33
E9	$CH_3O_2(\text{gas}) \rightleftharpoons CH_3O_2(\text{aq})$		2.0×10^3	6,600	E9=E1†
E10	$HNO_3(\text{gas}) \rightleftharpoons HNO_3(\text{aq})$		2.1×10^5	8,700	36
E11	$HNO_3(\text{aq}) \rightleftharpoons NO_3^- + H^+$		15.4		36
E12	$NO(\text{gas}) \rightleftharpoons NO(\text{aq})$		1.9×10^{-3}	1,480	36
E13	$NO_2(\text{gas}) \rightleftharpoons NO_2(\text{aq})$		6.4×10^{-3}	2,500	37
E14	$NO_3(\text{gas}) \rightleftharpoons NO_3(\text{aq})$		15.0		12
E15	$OH(\text{gas}) \rightleftharpoons OH(\text{aq})$		9.0×10^3		38
E16	$CO_2(\text{gas}) \rightleftharpoons CO_2(\text{aq})$		3.4×10^{-2}	2,420	39
E17	$CO_2(\text{aq}) \rightleftharpoons HCO_3^- + H^+$		4.5×10^{-7}	-1,000	39
Aqueous-phase reactions			K_{298}^*	$-E_a/R^\S$	Ref.
A1	$H_2O_2 + h\nu \rightarrow 2OH$		9.2×10^{-6}		
A2	$O_3 + h\nu + H_2O \rightarrow H_2O_2 + O_2$		3.2×10^{-5}		
A3	$CH_2(OH)_2 + OH + O_2 \rightarrow H_2O + HCOOH + HO_2$		2.0×10^9	-1,500	40, 41
A4	$HCOOH + OH + O_2 \rightarrow CO_2 + H_2O + HO_2$		1.6×10^8	-1,500	42
A5	$HCOO^- + OH + O_2 \rightarrow CO_2 + OH^- + HO_2$		2.5×10^9	-1,500	43
A6	$O_3 + O_2^- + H_2O \rightarrow OH + 2O_2 + OH^-$		1.5×10^9	-1,500	44, 45
A7	$HO_2 + O_2^- \rightarrow HO_2^- + O_2$		1.0×10^8	-1,500	46
A8	$HO_2^- + H^+ \rightarrow H_2O_2$		5.0×10^{10}	-1,500	2
A9	$H_2O_2 + OH \rightarrow HO_2 + H_2O$		2.7×10^7	-1,715	47
A10	$N_2O_5 + H_2O \rightarrow 2HNO_3$				†
A11	$CH_3O_2 + O_2^- + H_2O \rightarrow CH_3OOH + O_2 + OH^-$		5.0×10^7	-1,610	4
A12	$CH_3OOH + OH \rightarrow CH_3O_2 + H_2O$		2.7×10^7	-1,715	4
A13	$CH_3OOH + OH \rightarrow CH_2(OH)_2 + OH$		1.9×10^7	-1,860	4
A14	$HCO_3^- + OH \rightarrow H_2O + CO_3^{2-}$		1.0×10^7	-1,500	48
A15	$HCO_3^- + O_2 \rightarrow HO_2 + CO_3^{2-}$		1.5×10^6	-1,500	48
A16	$CO_3^{2-} + H_2O_2 \rightarrow HO_2 + HCO_3^-$		8.0×10^5	-2,800	49
A17	$CO_3^{2-} + O_2^- \rightarrow O_2 + CO_3^{2-}$		4.0×10^8	-1,500	49

G, E and A are gas-phase reactions, gas-liquid equilibrium and aqueous-phase reactions, respectively. These are the most important reactions from the more extended reaction scheme in the model. Some reactions summarize several reaction steps. Aqueous-phase processes at mid-latitudes in the Northern Hemisphere are simulated at pH 4.5, for other latitudes in Table 2 pH 5 is adopted⁶⁰. Photolysis rate coefficients and water vapour concentrations in clouds are kept equal to those during clear sky to emphasize photochemical differences. Daytime average photolysis rates are applied. Accommodation coefficients, as used for gas-to-aqueous-phase transfer processes¹⁴, are taken from refs 51–56. For those components with unknown accommodation coefficients we assume a value of 0.05.

* Reaction rate constants of first-order reactions are in s^{-1} , of second-order reactions in the gas phase (G) in $\text{molecule}^{-1} \text{cm}^3 \text{s}^{-1}$, in the aqueous phase (A) in $\text{mol}^{-1} \text{l s}^{-1}$. Photodissociation rate constants are calculated per season, latitude and altitude. The given values pertain to the Equator in July, at 700 mbar.

† K_{298} is in $\text{mol l}^{-1} \text{atm}^{-1}$ for Henry's law constants and in mol l^{-1} for aqueous-phase equilibria. The temperature dependence is calculated by $K = K_{298} \exp \{(-\Delta H/R)(1/T) - (1/298)\}$.

‡ Assumed.

§ E_a values are taken from ref. 4.

|| J.L., thesis in preparation.

* Gas-to-aqueous-phase transfer of N_2O_5 is limited by gas-phase diffusion and transfer through the interface. We assume that reaction A10 then occurs instantaneously, so that dissolution is irreversible.

TABLE 2 Initial conditions and concentrations used in the model

Case	Latitude	Altitude	T	t_c	t_{cf}	LWC	pH	NO _x	O ₃	CO	CH ₂ O	H ₂ O ₂	CH ₃ O ₂ H	OH	HO ₂
1	45° S	1.5	268	3.3	12.9	0.3	5.0	10	25	70	0.07	0.15	0.15	2.3×10^5	6.2×10^7
2	(winter)	3.0	260	3.7	14.9	0.2	5.0	10	25	65	0.06	0.15	0.15	2.5×10^5	6.1×10^7
3	45° S	1.5	285	3.2	14.4	0.3	5.0	7	15	50	0.2	0.6	0.75	9.4×10^5	1.8×10^8
4	(summer)	3.0	277	3.7	12.9	0.2	5.0	7	15	50	0.25	0.6	0.8	9.5×10^5	1.8×10^8
5	Equator	1.5	291	2.4	13.3	0.4	5.0	40	30	85	0.45	1.6	0.8	2.8×10^6	4.2×10^8
6		3.0	283	3.2	20.1	0.4	5.0	30	30	75	0.4	1.5	0.5	2.7×10^6	3.6×10^8
7		5.0	270	2.4	29.1	0.3	5.0	20	30	70	0.25	0.9	0.5	1.9×10^6	2.3×10^8
8	45° N	1.5	268	3.5	15.5	0.3	4.5	780	30	130	0.1	0.02	0.003	3.3×10^5	6.6×10^6
9	(winter)	3.0	260	3.9	16.3	0.2	4.5	400	30	115	0.15	0.04	0.008	5.3×10^5	1.7×10^7
10	45° N	1.5	285	3.0	16.2	0.3	4.5	135	40	90	0.45	1.0	0.2	3.5×10^6	3.5×10^8
11	(summer)	3.0	277	3.6	16.4	0.2	4.5	100	40	85	0.4	0.9	0.25	3.1×10^6	2.8×10^8

Altitudes are in km, temperatures in K. t_c and t_{cf} are the estimated mean periods in and outside clouds (in hours) for simulations in which clouds are considered²². LWC is the average liquid water content of the clouds (g m^{-3}) (ref. 22). Cloud-water pH values are based on ref. 50. Volume mixing ratios are given in p.p.b.v., except NO_x which is in p.p.t.v. Concentrations of OH and HO₂ are given in molecule cm^{-3} . NO_x, CO and O₃ mixing ratios (rounded to multiples of five here), as well as photolysis rates, have been taken from a global atmospheric chemistry model¹⁵. For the other species we list daytime average values, as obtained by simulations using our model, neglecting the influence of clouds.

production from reactions A3–5 and A9. HO₂ formation by reactions A3–5 and A9, in addition to gas-phase production (mainly through reaction G34), suffices to maintain O₃ destruction by reaction A6 at an appreciable rate for the entire residence time of air in clouds (≤ 4 h).

The prevailing reactions of HO₂ and O₂⁻ in the aqueous phase are either with O₃, with each other (A7) or with CH₃O₂ (A11), which yield OH, H₂O₂ and CH₃OOH, respectively. H₂O₂ formation in the aqueous phase is quite efficient (A7 + A8). Especially during the first hour after cloud formation, when OH still reacts predominantly with CH₂(OH)₂, cloud chemistry can be a source of H₂O₂. After this period, however, its breakdown by the fast reaction A9 can cause a net H₂O₂ concentration decrease. Whether clouds reduce the overall production of H₂O₂ in the troposphere also depends on the ambient NO_x concentrations. During cloudless conditions, when NO_x mixing ratios are ≤ 100 p.p.t.v., buildup of H₂O₂ in the gas phase is hardly impeded by competition for HO₂ by NO, so that H₂O₂ mixing ratios may reach up to 1.5 p.p.b.v. (parts per 10⁹ by volume) or more. Under these conditions, clouds are a net sink for H₂O₂ (apart from the aqueous-phase reaction of H₂O₂ with SO₂, which we do not consider here). When NO_x mixing ratios do exceed ~ 100 p.p.t.v., H₂O₂ production in clouds can, however, be more efficient as a result of the separation of NO from HO₂. Similarly, separation of CH₃O₂ from NO also promotes aqueous-phase formation of CH₃OOH by reaction A11. Because the solubility of CH₃OOH is much less than of H₂O₂, most of the CH₃OOH formed is released to the gas phase.

During the night, chemical activity in the atmosphere is low. A few reactions, however, are even favoured by darkness. Gaseous nitrate radicals (NO₃) are formed by reaction of NO₂ with

ozone (G23). During the day, NO₃ is rapidly photodissociated, but at night NO₃ can react with NO₂ to form N₂O₅ (G25). In the presence of cloud droplets, the heterogeneous reaction A10 may then generate significant amounts of HNO₃ (refs 10–12). This process is an important sink for NO_x, especially in a relatively cold environment, where thermal decomposition of N₂O₅ (reaction G26) is slow. For example, at 262 K reaction G26 is 100 times slower than at 298 K. Moreover, during the cold season at mid-latitudes nights are roughly twice as long as days. At the end of a night up to about one-half of the NO_x may be converted into N₂O₅. If condensation occurs, the accumulated N₂O₅ almost immediately forms HNO₃. Moreover, nighttime NO_x loss may be enhanced as a result of NO₃ scavenging by cloud droplets. In a cloudless situation NO₃ and N₂O₅ could be transformed into NO_x again during the next day by reactions G8, G9 and G11. Because photochemical processes involving NO_x as catalysts have a critical role in the formation of O₃ and OH during the day, a lowering of NO_x concentrations by clouds during the night is of great significance.

Cloud model and statistics

To demonstrate the impact of cloud chemical processes, we have developed a model (J.L., thesis in preparation) using a chemical scheme (Table 1) with the most essential reactions taking place in cloud-free background air and in non-precipitating clouds, which we assume to consist of droplets of 10- μm radius (only $\sim 10\%$ of clouds precipitate, the remainder evaporate¹³). We restrict our analysis to liquid water clouds. Besides gas- and aqueous-phase chemistry, which we model in a way similar to that in ref. 8, the model includes gas-aqueous and aqueous-phase mass transfer processes, using the formulation in ref. 14. A set of time-dependent first-order differential equations, based on rate expressions from ref. 14, is solved with variable step size. To simulate the diurnal cycle, photodissociation reactions are set to zero during the night. The same daytime average photodissociation coefficients (calculated using a global two-dimensional model¹⁵) are applied in both cloud-free air and in clouds, to distinguish between cloud chemical and radiative effects. Interestingly, calculations show that the average actinic fluxes for air parcels within clouds are approximately the same as those under cloud-free conditions (ref. 16 and S. Madronich, personal communication).

We used a global cloud distribution with a 10° latitudinal resolution (involving six cloud types) from refs 17 and 18. Combined with data about the approximate vertical extent of the different cloud types as function of latitude^{19,20}, we derived an average relative cloud coverage (volume fraction) for each atmospheric grid box (100 mbar $\times$ 10°). This coverage can be considered to be the fraction of time that air spends within clouds at the particular location—in the lower half of the troposphere air is apparently 'processed' by liquid water clouds for $\sim 15\%$ of the time. For the liquid water content of each cloud type we used the data in ref. 21.

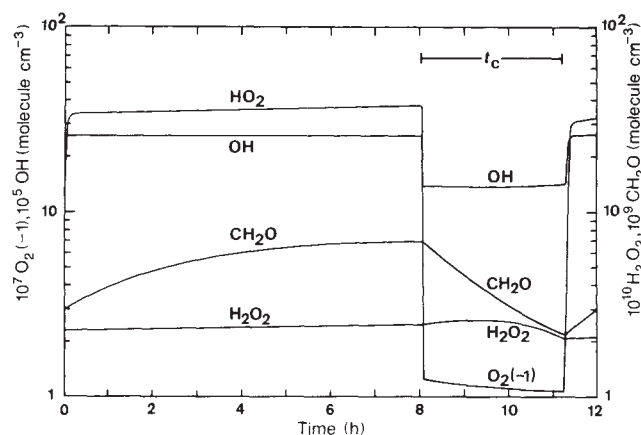


FIG. 2 Concentrations of some gases during sunlit hours of day 14 of the simulations, pertaining to the Equator at 3 km altitude (case 6, Table 2 and 3a), with NO_x fixed. For the cloudy period (t_c) total gas-phase plus aqueous-phase concentrations are depicted. $\text{O}_2(-) = \text{HO}_2(\text{gas}) + \text{HO}_2(\text{aq}) + \text{O}_2^-$.

TABLE 3 Model-calculated parameters for selected latitudes, seasons and heights

(a) Constant NO_x mixing ratios

Case	Latitude	Altitude	$r_{\text{CH}_2\text{O}}$	$r_{\text{H}_2\text{O}_2}$	r_{OH}	r_{HO_2}	$\frac{d}{dt}[\text{O}_3]_{\text{nc}}$	$\frac{d}{dt}[\text{O}_3]_{\text{c}}$
1	45° S	1.5	-55	-45	-30	-35	-0.01	-0.04
2	(winter)	3.0	-70	-60	-40	-45	-0.02	-0.03
3	45° S	1.5	-25	-20	-15	-15	-0.4	-0.6
4	(summer)	3.0	-30	-25	-15	-20	-0.3	-0.4
5	Equator	1.5	-30	-20	-15	-20	-0.3	-0.7
6		3.0	-25	-15	-15	-15	-0.4	-0.8
7		5.0	-15	-15	-10	-10	-0.2	-0.3
8	45° N	1.5	-55	+45	-35	-40	+0.4	+0.3
9	(winter)	3.0	-60	+65	-30	-35	+0.8	+0.5
10	45° N	1.5	-25	≈0	-15	-15	+3.3	+2.3
11	(summer)	3.0	-30	-5	-15	-20	+3.0	+2.1

(b) Heterogeneous loss of N₂O₅ in clouds included

Case	Latitude	Altitude	r_{NO_x}	$r_{\text{CH}_2\text{O}}$	$r_{\text{H}_2\text{O}_2}$	r_{OH}	r_{HO_2}	$\frac{d}{dt}[\text{O}_3]_{\text{nc}}$	$\frac{d}{dt}[\text{O}_3]_{\text{c}}$
1	45° S	1.5	-80	-70	-60	-55	-60	-0.01	-0.08
2	(winter)	3.0	-85	-75	-65	-60	-70	-0.02	-0.05
3	45° S	1.5	-30	-35	-30	-25	-30	-0.4	-0.7
4	(summer)	3.0	-35	-40	-35	-30	-35	-0.3	-0.5
5	Equator	1.5	-25	-35	-30	-25	-30	-0.3	-1.1
6		3.0	-25	-30	-25	-20	-25	-0.4	-1.0
7		5.0	-20	-20	-25	-20	-25	-0.2	-0.6
8	45° N	1.5	-85	-35	+320	-50	+10	+0.4	+0.3
9	(winter)	3.0	-65	-45	+110	-50	-40	+0.8	+0.4
10	45° N	1.5	-20	-30	-5	-20	-25	+3.3	+1.8
11	(summer)	3.0	-25	-35	-15	-25	-30	+3.0	+1.6

$r_{\text{CH}_2\text{O}}$, $r_{\text{H}_2\text{O}_2}$, r_{OH} and r_{HO_2} represent the average percentages reduction (–) or increase (+) in the concentrations of these gases, comparing simulations with and without clouds, averaging the concentrations over the last 5 days of 15 days of simulation. The numbers have been rounded to 5%. $d[\text{O}_3]_{\text{nc}}/dt$ represents the daytime average production (+) or destruction (–) rate of O₃ in p.p.b.v. per day, for simulations in which the influence of clouds is neglected. $d[\text{O}_3]_{\text{c}}/dt$ represents a similar rate for simulations including clouds. Two examples (case 6 and 10, constant NO_x) of how these figures were obtained are given in Table 4.

In *b*, the NO_x mixing ratios in simulations without clouds are kept constant at the indicated values (Table 2), because we assume that in this case transport and chemical loss processes are in equilibrium. The same NO_x source due to transport processes has been adopted in the simulations which include cloud processes. $d[\text{O}_3]_{\text{nc}}/dt$ here, therefore, is equal to $d[\text{O}_3]_{\text{c}}/dt$ in *a*.

The times that the air spends in clouds (t_{c}) relative to that spent in cloud-free (t_{cf}) conditions are important quantities determining the effects of cloud chemical processes. In ref. 22 these have been estimated on the basis of the cloud coverage, the estimated typical heights and updraft velocities of air through convective clouds, and lifetime estimates of stratiform clouds^{23,24}. This analysis indicates that, on the average, periods of ~2–4 h in clouds are followed by cloud-free periods that are 5–20 times longer. For the simulations the clouds are 'turned on and off' for periods appropriate to the global location. To study the steady-state effect of clouds on the atmospheric photochemistry, we simulated 15 days in succession, starting with initial conditions obtained with a global, time-dependent, meridional two-dimensional model¹⁵ that does not treat cloud chemical processes. We compare model results with and without the chemical effects of clouds, averaging the results over the last five days of the simulations.

Our approach involves a coarse treatment of the occurrence and the radiative and microphysical properties of clouds. 'Average' clouds do not exist, and air parcels do not move into and out of clouds at the same altitude, as we have assumed. Here we merely intend to illustrate the importance of cloud chemical processes in global atmospheric chemistry.

Results and discussion

Results of the calculations—which we have performed for conditions prevailing at the Equator and at mid-latitudes (as adopted from the two-dimensional model, see Table 2), in the Northern and Southern Hemisphere, during summer and winter and for different altitudes—are summarized in Tables 3 and 4. We compare the results of model simulations with and without cloud

chemistry. To separate chemical from radiative effects, we kept the ozone concentrations fixed at the values specified in Table 2. Despite this assumption, the CH₂O, H₂O₂ and HO_x concentrations as well as net O₃ production rates, are in most situations considerably lowered by clouds. Table 3*a* shows the effect of cloud chemistry in simulations during which we kept the NO_x mixing ratios constant. The results presented in Table 3*b* pertain to simulations that included the NO_x loss caused by the nighttime conversion of N₂O₅ to HNO₃ in clouds. In the latter case we applied a local NO_x production term, equal to the chemical loss rate of NO_x by reaction G18, which we calculated in the simulations without clouds and for fixed NO_x. Gas-phase chemical loss of NO_x was thus balanced by a term that simulated the combined effects of transport from source areas and production by lightning. The results indicate that nighttime NO_x destruction by cloud processes (through A10) cause NO_x concentration decreases of 20–85%. The largest depletions occur during the winter, when the effect of reaction A10 dominates over that of reaction G18.

Cloud chemical effects reduce the OH and HO₂ concentrations by 10–70% (except case 8, Table 3*b*). During summer and at the Equator, HO_x reductions are generally in the range 15–30% if N₂O₅ scavenging is neglected, or 20–50% if we take it into account. The general loss of HO_x radicals, and consequently of H₂O₂, is mainly the result of the rapid oxidation of CH₂O in clouds and the resulting decrease in HO_x production by reaction G5. Aqueous-phase loss of H₂O₂ (A9) is also an important HO_x sink. As an example, the HO_x fluxes calculated for situations without and with clouds are depicted in Fig. 1, which pertains to conditions at the Equator at an altitude of about 3 km (case 6, Tables 2 and 3*a*). Figure 1*b, c* shows that,

TABLE 4 Ozone production and destruction rates

(a) At the Equator, at an altitude of 3 km (case 6, Tables 2 and 3a)

		Simulations without clouds	Simulations with clouds	
Reaction			t_{cf}	t_c
O ₃ production	NO + HO ₂	5.3	5.2	1.5
	NO + CH ₃ O ₂	2.6	2.5	0.4
O ₃ destruction	O(¹ D) + H ₂ O	5.2	5.2	5.2
	HO ₂ + O ₃	3.6	3.6	0.1
	OH + O ₃	0.8	0.8	0.4
	O ₂ ⁻ + O ₃ (aq)	—	—	7.1
Net O ₃ destruction		1.7	1.9	12.3
Weighted average net O ₃ destruction for $t_{cf} + t_c$			3.3	

(b) At mid-latitudes in the Northern Hemisphere during summer, altitude 1.5 km (case 10, Tables 2 and 3a)

		Simulations without clouds	Simulations with clouds	
Reaction			t_{cf}	t_c
O ₃ production	NO + HO ₂	19.3	19.1	1.0
	NO + CH ₃ O ₂	5.9	6.0	1.7
O ₃ destruction	O(¹ D) + H ₂ O	4.3	4.3	4.3
	HO ₂ + O ₃	6.1	6.0	0.3
	OH + O ₃	1.9	1.9	1.0
	O ₂ ⁻ + O ₃ (aq)	—	—	7.0
Net O ₃ production		12.9	12.8	-9.9
Weighted average net O ₃ production for $t_{cf} + t_c$			9.3	

Rates are in 10^5 molecule $\text{cm}^{-3} \text{s}^{-1}$. NO_x was constant during these simulations. t_{cf} represents the cloud-free period and t_c the cloudy period.

1.5 h after cloud formation in the air parcel, there is still a slight buildup of H₂O₂. After some 2 h, however, when CH₂(OH)₂ is further depleted (by A3), the OH level in the droplets rises, so that reaction A9 becomes more important as a source of HO₂.

Although the absolute effect of clouds on HO_x chemistry is most important in the summer and in the tropics, the relative effect is strongest in winter—the aqueous-phase processes are favoured by more efficient dissolution in the cold season, and the low photochemical activity (compared with summer and the tropics) prevents a recovery of the CH₂O, HO_x and H₂O₂ levels between successive cloud events to the levels that are observed during simulations without clouds.

In the industrialized mid- and high latitudes of the Northern Hemisphere during winter, when NO_x concentrations are highest, the nighttime NO_x loss can be so large that an increase in HO₂ concentrations occurs because of a reduction of the rate of reaction G22 aided by a decreased HO_x destruction through reaction G18. This leads to an enhanced buildup of H₂O₂ through reaction G21 in cloud-free periods. Moreover, in these NO_x-rich environments, partial separation of HO₂ from NO causes H₂O₂ production to be more efficient inside clouds, which contributes to the H₂O₂ increase. Note, however, that in these situations (cases 8 and 9, Tables 2 and 3) H₂O₂ concentrations are quite low.

The most surprising outcome of our study is the strong effect of cloud chemistry on the tropospheric O₃ budget. To a large extent this is the result of the effective chemical breakdown of O₃ through reaction A6. This, in turn, is due to replenishment of O₂⁻ by uptake of HO₂ from the gas phase and recycling reactions A3–5, A9 and E2. Net O₃ destruction is further enhanced by a reduction of O₃ formation in clouds (through the separation of NO from HO₂ and CH₃O₂), as well as during cloud-free periods (through the reduction of NO_x and HO_x concentrations). Figures 1 and 2 show the mean chemical fluxes and concentrations, respectively, for a selected location near

the Equator. The corresponding O₃ production and destruction rates are shown in Table 4a. A similar example, pertaining to mid-latitudes in the Northern Hemisphere, is given in Table 4b.

The resulting net average O₃ fluxes for all simulations that include the effects of clouds are given in the last columns of Table 3a, b. We observe that, when there are no clouds, net photochemical O₃ production occurs mainly over the Northern Hemisphere, whereas net destruction is more important over the Southern Hemisphere and at the Equator. The balance is a result of transport processes. When clouds are introduced, net O₃ destruction rates during summer in the NO_x-poor areas are enhanced by factors ranging from 1.3–2.3 if N₂O₅ scavenging is neglected, and 1.7–3.7 if it is included. Photochemical O₃ formation rates in NO_x-rich areas are reduced by ~35% and ~45%, respectively. The largest effects are calculated for the Northern Hemisphere summer and for the tropics. This is due to the relatively high HO_x and CH₂O concentrations in these regions, which are required to destroy O₃ and recycle O₂⁻ in the aqueous phase. In the tropics, moreover, clouds have the highest liquid water contents and relatively low acidity. The pH is a controlling factor because it influences the equilibrium between HO₂ and O₂⁻ (E2). Therefore, acidification of cloud water (by sulphuric acid or ammonium bisulphate, for example) can 'protect' O₃, in the sense that at low pH less O₃ is destroyed by O₂⁻.

In spite of our simplified treatment of cloud physical processes, we conclude that clouds can have a large influence on the photochemistry of the troposphere, reducing concentrations of O₃, NO_x and HO_x in particular, and thus affecting the tropospheric oxidation efficiency and trace-gas budgets. For instance, the production rates of H₂ and CO through CH₂O breakdown are reduced by similar percentages as the reductions of CH₂O concentrations (15–70%). On the other hand, destruction of H₂ and CO by OH is also decreased (20–60%). This implies that the relative importance of CO and H₂ sources other than CH₄ (and other hydrocarbon) oxidation increases considerably. If we had allowed O₃ to vary throughout our simulations, calculated O₃ concentrations would have become much lower, leading to even lower OH concentrations. In this case, however, the calculated O₃ concentrations would have been markedly lower than observed. The solution to this discrepancy could be larger O₃ fluxes from the stratosphere, more NO_x production by lightning or more efficient transfer of NO_x-rich boundary-layer air to the free troposphere by convection²⁵, which would enhance the photochemical O₃ production efficiency²⁶. In any case, our study indicates that photochemical processes in clouds can lead to strong reductions in the concentrations of O₃, CH₂O, NO_x and HO_x in the troposphere. We conclude, therefore, that advanced global-scale tropospheric chemistry modelling requires development of routines that properly simulate cloud occurrence and cloud chemistry. □

Received 25 September; accepted 1 December 1989.

- Calvert, J. G. *et al.* *Nature* **317**, 27–35 (1985).
- Graedel, T. E. & Weschler, C. J. *Rev. Geophys. Space Phys.* **19**, 505–538 (1981).
- Chameides, W. L. & Davis, D. D. *J. geophys. Res.* **87**, 4863–4877 (1982).
- Jacob, D. J. *J. geophys. Res.* **91**, 9807–9826 (1986).
- Crutzen, P. J. *A. Rev. Earth planet. Sci.* **7**, 443–472 (1979).
- Atmospheric Ozone Rep. 16* (World Meteorological Organisation, Geneva, 1985).
- Conrad, R. & Seiler, W. *J. geophys. Res.* **85**, 5493–5498 (1980).
- Chameides, W. L. *J. geophys. Res.* **89**, 4739–4755 (1984).
- Hartmann, W. R., Andreae, M. O. & Helas, G. *Atmos. Environ.* **23**, 1531–1533 (1989).
- Platt, U., Perner, D., Schröder, J., Kessler, C. & Toennissen, A. *J. geophys. Res.* **86**, 11965–11970 (1981).
- Heikes, B. G. & Thompson, A. M. *J. geophys. Res.* **88**, 10883–10895 (1983).
- Chameides, W. L. *J. geophys. Res.* **91**, 5331–5337 (1986).
- Pruppacher, H. R. & Klett, J. D. *Microphysics of Clouds and Precipitation* (Reidel, Dordrecht, 1980).
- Schwartz, S. E. in *NATO, Adv. Sci. Ser. Vol. G6* (ed. Jaeschke, W.) 415–471 (1986).
- Crutzen, P. J. & Gidel, L. T. *J. geophys. Res.* **88**, 6641–6661 (1983).
- Madronich, S. *J. geophys. Res.* **92**, 9740–9752 (1987).
- Hahn, C. J., Warren, S. G., London, J., Chervin, R. M. & Jenne, R. *Natn. Cent. Atmos. Res., Tech. Note TN-201 + STR* (NCAR, Boulder, 1982).
- Warren, S. G., Hahn, C. J., London, J., Chervin, R. M. & Jenne, R. *Natn. Cent. Atmos. Res., Tech. Note TN-273 + STR* (NCAR, Boulder, 1986).
- Paltridge, G. W. & Platt, C. M. R. *Radiative Processes in Meteorology and Climatology* (Elsevier, Amsterdam, 1976).
- Telegadas, K. & London, J. *Air Force Sci. Rep.* 19 (122)–165 (New York University, 1954).

21. Mason, B. J. *The Physics of Clouds* (Clarendon, Oxford, 1971).
22. Lelieveld, J., Crutzen, P. J. & Rodhe, H. *GLOMAC 89/1, Rep. CM-76* (Int. Met. Inst., University of Stockholm, 1989).
23. Atkinson, B. W. *Mesoscale Atmospheric Circulations* (Academic, London, 1981).
24. Matveev, L. T. *Cloud Dynamics* (Reidel, Dordrecht, 1984).
25. Chatfield, R. B. & Crutzen, P. J. *J. geophys. Res.* **89**, 7111–7132 (1984).
26. Pickering, K. E. *et al.* *J. geophys. Res.* (submitted).
27. DeMore, W. B. *et al.* *Jet Propulsion Lab. Publ.* 87-41 (NASA-JPL, Pasadena, 1987).
28. Baulch, D. L. *et al.* *J. Phys. Chem. Ref. Data* **13**, 1259–1379 (1984).
29. Jenkin, M. E., Cox, R. A., Hayman, G. D. & White, L. J. *JCS Faraday Trans. II* **84**, 913–930 (1988).
30. Vaghjani, G. L. & Ravishankara, A. R. *J. phys. Chem.* **93**, 1948–1959 (1989).
31. Schwartz, S. E. *J. geophys. Res.* **89**, 11589–11598 (1984).
32. Perrin, D. D. *Ionization Constants of Inorganic Acids and Bases in Aqueous Solution* (Pregamon, New York, 1982).
33. Lind, J. A. & Kok, G. L. *J. geophys. Res.* **91**, 7889–7895 (1986).
34. Kozac-Channing, L. F. & Heltz, G. R. *Environ. Sci. Technol.* **17**, 145–149 (1983).
35. Weast, R. C. (ed.) *Handbook of Chemistry and Physics* 67th edn (Chemical Rubber Company, Boca Raton, 1986).
36. Schwartz, S. E. & White, W. H. *Adv. Environ. Sci. Eng.* **4**, 1–45 (1981).
37. Lee, Y.-N. & Schwartz, S. E. *J. geophys. Res.* **86**, 11971–11983 (1981).
38. Chang, J. S. *Natn. Cent. Atmos. Res. Tech. Note TN-256 + SRT* (NCAR, Boulder, 1985).
39. Smith, R. M. & Martell, A. E. *Critical Stability Constants* Vol. 4 (Plenum, New York, 1976).
40. Markovic, V. & Sehested, K. *Proc. Tihany Symp. Radiat. Chem.* **3**, 1243–1253 (1972).
41. Bothe, E. & Schulte-Frohlinde, D. *Z. Naturf.* **35b**, 1035–1039 (1980).
42. Scholes, G. & Wilson, R. L. *JCS Faraday Trans.* **63**, 2982–2993 (1967).
43. Anbar, M. & Neta, P. *Int. J. appl. Radiat. Isotopes* **18**, 493–523 (1967).
44. Buhler, R. E., Staehelin, J. & Hoigne, J. *J. phys. Chem.* **88**, 3560–3564 (1984).
45. Sehested, K., Holcman, J. & Hart, E. J. *J. phys. Chem.* **87**, 1951–1954 (1983).
46. Bielski, B. H. J. *Photochem. Photobiol.* **28**, 645–649 (1978).
47. Christensen, H., Sehested, K. & Corfitzen, H. *J. phys. Chem.* **86**, 1588–1590 (1982).
48. Farhatziz & Ross, A. B. *Rep. NSRDS-NBS 59* (Natn. Bureau of Standards, Washington, DC, 1977).
49. Behar, D., Czapski, G. & Duchovny, I. *J. phys. Chem.* **74**, 2206–2210 (1970).
50. Ogren, J. & Rodhe, H. *Tellus* **B38**, 190–196 (1986).
51. Lee, J. H. & Tang, I. N. *Atmos. Environ.* **22**, 1147–1151 (1988).
52. Tang, I. N. & Lee, J. H. in *Am. Chem. Soc. Symp. Ser.* Vol. 349 (eds Johnson, R. W. & Gordon, G. E.) 109–117 (Am. Chem. Soc., Washington, 1986).
53. Mozurkewich, M. & Calvert, J. G. *J. geophys. Res.* **93**, 15889–15896 (1988).
54. Mozurkewich, M., McMurry, P. H., Gupta, A. & Calvert, J. G. *J. geophys. Res.* **92**, 4163–4170 (1987).
55. Worsnop, D. R. *et al.* *J. phys. Chem.* (in the press).
56. Gardner, J. A. *et al.* *J. geophys. Res.* **92**, 10887–10895 (1987).

ACKNOWLEDGEMENTS. We thank K. M. Valentin for her assistance with the MPI two-dimensional atmospheric chemistry model. J.L. is supported by the Bundesministerium für Forschung und Technologie (FRG) through the EUROTRAC subproject EUMAC.

Regulation of microtubule dynamics by cdc2 protein kinase in cell-free extracts of *Xenopus* eggs

Fulvia Verde, Jean-claude Labbé*, Marcel Dorée* & Eric Karsenti

European Molecular Biology Laboratory, Cell Biology Program, Meyerhofstrasse 1, D-6900, Heidelberg, FRG

Microtubules are involved in the transport of vesicles in interphase and of the chromosomes during mitosis. Their arrangement and orientation in the cell are therefore of prime importance and specific patterns are believed to be generated by modulations of the intrinsic dynamic instability of microtubules. Here it is shown that the interphase–metaphase transition of microtubule arrays is under the control of the cdc2 kinase that precisely regulates the dynamics and steady-state length of microtubules.

IN the cell cycle, the transition from interphase to mitosis involves a dramatic reorganization of the whole cell. Of special interest are the changes in the microtubule network leading to the assembly of the mitotic apparatus which accurately segregates the genetic material between the two daughter cells^{1,2}. Assembly of the mitotic spindle proceeds through a complex sequence of events involving centrosome duplication and separation³, increased nucleation of the centrosomes⁴, disassembly of cytoplasmic microtubules associated with increased microtubule turnover^{5–8}, connection of the kinetochores to the poles by microtubule bundles^{9–11}, and lateral interactions between microtubules in the central region of the mitotic spindle¹². Among these events, the regulation of microtubule dynamics seems to be a key process^{11,13}. *Xenopus* egg extracts have proved suitable for studying the regulation of microtubule dynamics. Extracts prepared from eggs during interphase of the first embryonic cell cycle support the growth of microtubules from centrosomes as *in vivo*^{14–18}, and promote mitotic spindle assembly on sperm nuclei in response to the addition of partially purified maturation promoting factor (MPF)¹⁹. This factor has been recently purified from *Xenopus* and starfish eggs^{20,21}. It is a

complex of cyclin and cdc2 kinase (p 34) in its active form²². By using *Xenopus* egg extracts, isolated centrosomes, and active cdc2 kinase purified from starfish eggs^{21–24}, we show that the changes in microtubule dynamics occurring at the interphase–metaphase transition are regulated by cdc2 kinase.

Microtubule elongation and steady-state length

The egg extracts that we used were prepared according to Lohka and Maller¹⁹ with some buffer modifications²⁵. We prepared metaphase extracts directly from nonactivated *Xenopus* eggs (naturally arrested in metaphase II of meiosis). We considered these extracts as truly metaphasic only when stable cdc2 kinase activity was higher than 8 pmol phosphate transferred from ATP to H1 histones per min per μ l extract. Such extracts have high MPF activity and a pattern of protein phosphorylation similar to that found *in vivo*²⁵. We prepared interphase extracts from eggs released from arrest in metaphase II by an electric shock¹⁴. In these extracts, the histone kinase activity remained low for several hours of incubation at room temperature. The protein concentration was ~ 40 mg ml⁻¹ and the tubulin concentration ~ 12 μ M. Incubation of interphase extracts for 15 min at room temperature resulted in the spontaneous assembly of many long microtubules from tubulin (Fig. 1). By contrast, we found only a few short microtubules in metaphase extracts incubated for the same time at room temperature.

Purified centrosomes can organize polymerization of tubulin into microtubules. Addition of purified centrosomes to the interphase extracts resulted in the assembly of very long microtubules as early as 3 min after the beginning of the incubation at room temperature when there was still little spontaneous polymerization. By contrast, addition of purified centrosomes to the metaphase extracts nucleated the assembly of microtubules that remained short over 30 min of incubation at room temperature. In interphase extracts, microtubules grew at an initial rate of 18 μ m min⁻¹, stopped growing after they reached 30–65 μ m at 3 min, and shrunk to a stable mean value of ~ 20 μ m (Fig. 2). This was probably the result of depletion of the soluble tubulin pool due to extensive spontaneous polymerization. In mitotic extracts, the initial elongation rate was much slower

* CNRS and INSERM, BP 5051, 34033 Montpellier Cedex, France

($3.8 \mu\text{m min}^{-1}$), and microtubules stopped growing after they reached a steady-state length of 7–12 μm . We calculated that when centrosome-nucleated microtubules are shorter than 100 μm , they should not affect the free tubulin concentration in the mitotic extracts. Depletion of free tubulin subunits cannot therefore explain the short steady-state length of the microtubules in metaphase extracts. Another parameter determining the stability of microtubules is the concentration of nucleotide. We therefore measured the ATP and GTP concentration in these extracts during their incubation at room temperature by separating them on a strong ion-exchange column. The ATP and GTP concentrations remained constant at 1.5 mM and 0.5 mM, respectively, for at least 30 min. Nucleotide depletion cannot therefore account for the short microtubule length in mitotic extracts. There must be a specific mechanism that prevents microtubules from growing any more in metaphase extracts.

Microtubule dynamics and cdc2 kinase

We expected the difference between the specific protein contents of interphase and metaphase extracts to be negligible because we obtained interphase eggs by simple activation of eggs arrested in metaphase II, and no protein synthesis is required for this transition. The transition from interphase to mitosis requires only the synthesis of cyclin^{26,27}, which allows cdc2 kinase to be activated by dephosphorylation^{21,28,29}. The difference in microtubule dynamics between interphase and mitotic extracts is therefore likely to be due to the enzymatic activity of the cdc2 kinase.

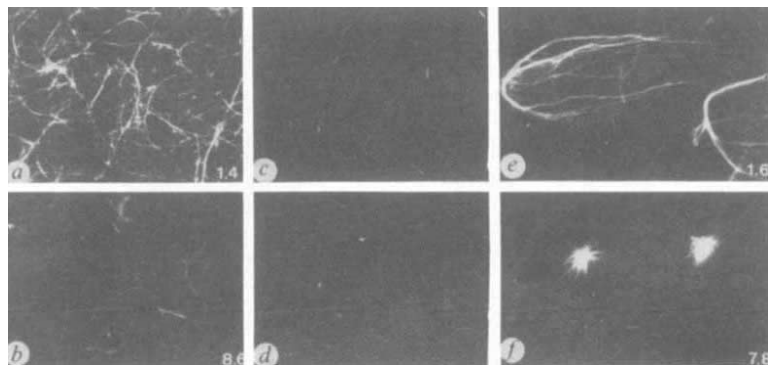
We added centrosomes to an interphase extract and let the microtubules grow for 10 min at room temperature before adding the purified cdc2 kinase (Fig. 3). Two min after addition of the kinase, the free microtubules disappeared. Five min after kinase addition, centrosome-nucleated microtubules started and continued to shrink until they reached a stable length of $\sim 7 \mu\text{m}$ (Fig. 3, 15 min). In this experiment, the kinase activity in the extract at the time of microtubule fixation was close to physiological mitotic levels³⁰. We then determined the elongation rate of microtubules in interphase extracts treated with cdc2 kinase (Fig. 4). We pre-incubated the interphase extract with or without purified cdc2 for 15 min at room temperature before we added the centrosomes. The initial elongation rate dropped from

20 $\mu\text{m min}^{-1}$ in the untreated extract to 4 $\mu\text{m min}^{-1}$ in the cdc2-treated extract. Also the mean microtubule steady-state length was reduced from $\sim 22 \mu\text{m}$ in the untreated extract to 7–8 μm in the cdc2-treated extract. Therefore we had exactly reproduced the microtubule dynamics of metaphase-II egg extracts by treating interphase extracts with starfish cdc2 kinase (compare Figs 2 and 4c). At the beginning of the incubation with cdc2-kinase, the centrosomes were weakly phosphorylated, as revealed by the MPM2 monoclonal antibody directed against phosphorylated epitopes^{31,32}, whereas later they became brightly stained by MPM2 (Fig. 4d, e). This did not occur in the absence of cdc-2 kinase (data not shown). The centrosomes started to nucleate many more microtubules at the same time as they showed increased staining by the MPM2 antibody (Fig. 4, second row). This indicates that phosphorylation of centrosomes actually increases their nucleating activity, and we are now investigating this point further. The cdc2 kinase can be purified in two different ways, the final preparation containing either cdc2 and cyclin B as a pure 1:1 stoichiometric complex²², or cdc2 as the main polypeptide²³ but with no intact cyclin. We observed no difference between the effects of the two preparations on microtubule dynamics. As a specificity control, we tested the effect of the catalytic subunit of the cyclic AMP-dependent protein kinase. This enzyme had no effect on the elongation rate or mean length of microtubules at steady state, even at levels of high activity (data not shown).

The above results strongly indicate that cdc2 kinase induces microtubules to destabilize in a way similar to that observed at the onset of mitosis *in vivo*^{7,8,33}. We examined this possibility by testing the sensitivity to nocodazole of microtubules assembled in extracts with and without cdc2 (Fig. 5). We incubated interphase extracts containing centrosomes for 15 min at room temperature in the absence or presence of cdc2 kinase to allow microtubule assembly to reach a steady state (Fig. 5a, d). We then added, nocodazole (20 μM) and examined the kinetics of microtubule depolymerization. The free microtubules in interphase extracts disappeared between 7 and 10 min after the addition of nocodazole. During this time the centrosome-nucleated microtubules remained perfectly stable (Fig. 5b), finally disappearing between 10 and 20 min after the addition of nocodazole (Fig. 5c). In cdc2-treated extracts, microtubules

FIG. 1 Microtubule polymerization in frog extracts. Spontaneous tubulin polymerization after a 15-min incubation at room temperature of interphase extracts prepared from eggs sampled 20 min after activation (a) and metaphase-II extracts (b). Centrosome-induced microtubule assembly after a 0-min (c) and a 3-min (e) incubation in an interphase extract, and after a 0-min (d) and a 15-min (f) incubation in a metaphase-II extract. No exogenous tubulin was added to the extracts. Histone kinase activity in the extract at the time of fixation is given in the lower right corner of each figure in pmol phosphate transferred onto histones per min per μl extract. Magnification, $\times 825$.

METHODS. Supernatants of centrifugations at 100,000g were prepared from interphase and metaphase-II extracts and stored in liquid nitrogen as described by Felix *et al.*²⁵. The buffer used to prepare the extract contained 100 mM potassium acetate, 2.5 mM magnesium acetate, 60 mM EGTA, 5 $\mu\text{g ml}^{-1}$ cytochalasin D, 1 mM DTT (pH 7.2). Packed eggs were crushed by centrifugation at 10,000g for 15 min. in a minimum volume of buffer. This buffer was diluted $\sim$ threefold by the cytoplasm. An ATP regenerating system (1 mM ATP, 10 mM creatin phosphate, 80 $\mu\text{g ml}^{-1}$ creatine phosphokinase, final concentration) was added to the extract before further centrifugation at 100,000g. These extracts were still contaminated by particulate and membranous material. Protein concentration was 30–40 mg ml^{-1} and tubulin concentration 8–12 μM . Centrosomes were prepared from KE 37 lymphoid cells according to Bornens *et al.*⁴⁶. Microtubule polymerization was studied in 15- μl aliquots of extracts incubated at room temperature without or with centrosomes (10^3 per μl extract). Microtubules were visualized by immunofluorescence after fixation of the extracts by dilution in 1 ml of 0.25% glutaraldehyde in RG1 medium (80 mM PIPES buffer, 1 mM EGTA, 1 mM MgCl_2 , 1 mM GTP, pH 6.8 with KOH). After 6 min at room



temperature, the suspension was layered on a 5-ml cushion of 25% glycerol (v/v) in RG2 medium (RG1 without GTP) in 15-ml corex tubes and centrifuged on a coverslip as described by Evans *et al.*⁴⁷. The coverslips were postfixed in methanol at -20°C for 5 min and treated with freshly prepared 0.1% sodium borohydride (Sigma, S-9125) in PBS for 10 min at room temperature. Immunofluorescence was performed with a polyclonal rabbit anti-tubulin antibody and fluorescein-conjugated goat anti-rabbit antibody, diluted in PBS containing 3% BSA and 0.1% NaN_3 . The coverslips were mounted in Moewiol, and photographs were taken using a Neofluar 100X lens, mounted on a Zeiss Axiophot Photomicroscope, and recorded on Kodak TMY-400 films. ATP and GTP were quantitated in the extracts at the beginning and end of each experiment as described in ref. 30. We found the same values as *in vivo*³⁰.

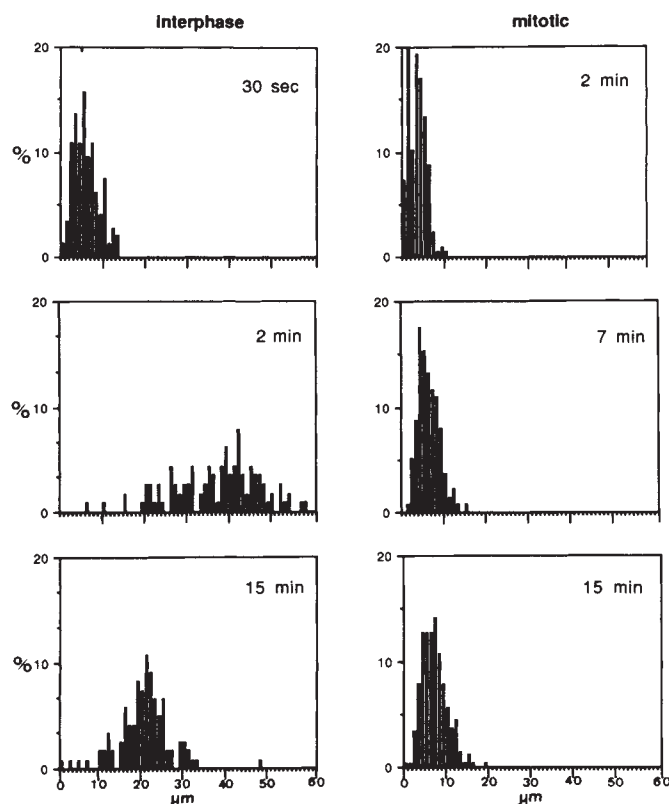


FIG. 2 Elongation rate of centrosome-nucleated microtubules in interphase and metaphase-II extracts. Centrosomes were mixed with the extracts at 4 °C and samples were fixed after the indicated times of incubation at room temperature. Immunofluorescence was carried out as described in Fig. 1. Microtubule length was determined on the negative enlarged on a video screen, using a computer program. Between 100 and 200 microtubules were measured for each sample. The histograms show the microtubule length distribution in interphase and mitotic extracts after the indicated time of incubation at room temperature. The curves show the increase in microtubule mean length with time, in interphase (open squares) and metaphase-II extracts (closed symbols). The mean length was obtained by dividing the total polymer length by the number of individual microtubules measured.

remained intact at 1 min (Fig. 5d), started to shrink at 2 min (data not shown) and were absent by 2.5 min (Fig. 5e) after the addition of nocodazole. These results compare directly with the *in vivo* effect of nocodazole during the cell cycle³³, and indicate that in interphase extracts, the half-life of free microtubules is ~3–5 min, whereas the half-life of centrosome-nucleated microtubules is about 10 min. This strongly indicates that during interphase, the plus ends of microtubules are quite stable and

that free microtubules depolymerize from their minus ends. In the cdc2-treated extracts, the half-life of centrosome-nucleated microtubules is ~1 min. This is probably because their plus ends are extremely unstable.

Microtubule dynamics and phosphorylation

Addition of cdc2 kinase with centrosomes to an interphase extract resulted in a fast elongation of centrosome-nucleated

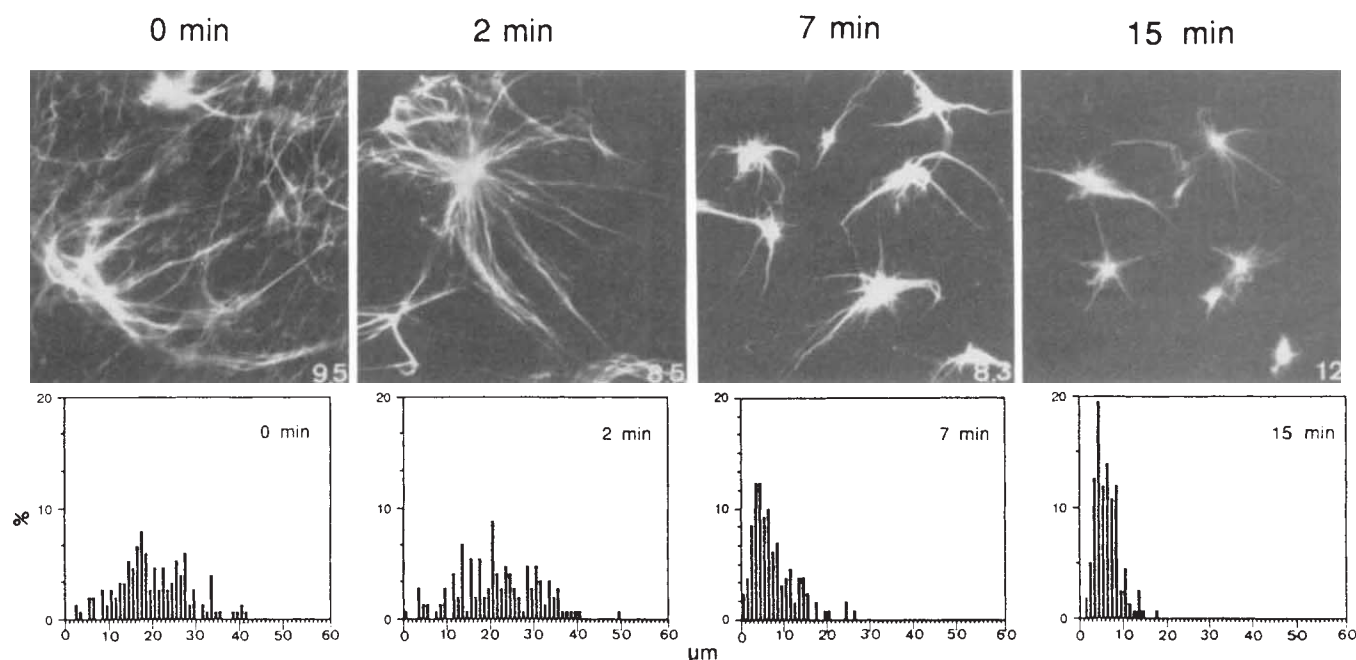


FIG. 3 Effect of cdc2 kinase on microtubule length in interphase extracts. Centrosomes were pre-incubated in an interphase extract for 10 min at room temperature to assemble interphasic asters. Cdc2 kinase (>95% pure, 150 $\mu\text{g ml}^{-1}$ stock solution²²) was then added, and samples were fixed, both for immunofluorescence and histone kinase assay at the indicated times. Changes in microtubule length are shown on the pictures and quantified in the histograms. Histone kinase activity in the extract at each time point is shown in the right lower corner of each picture (in $\text{pmol min}^{-1} \mu\text{l}^{-1}$). The interphase extract used in this experiment was obtained from eggs homogenized 20 min after activation by an electric shock¹⁴. The cdc2 kinase (1 μl) was added to 15- μl aliquots of extracts. Magnification, $\times 825$.

tated in the histograms. Histone kinase activity in the extract at each time point is shown in the right lower corner of each picture (in $\text{pmol min}^{-1} \mu\text{l}^{-1}$). The interphase extract used in this experiment was obtained from eggs homogenized 20 min after activation by an electric shock¹⁴. The cdc2 kinase (1 μl) was added to 15- μl aliquots of extracts. Magnification, $\times 825$.

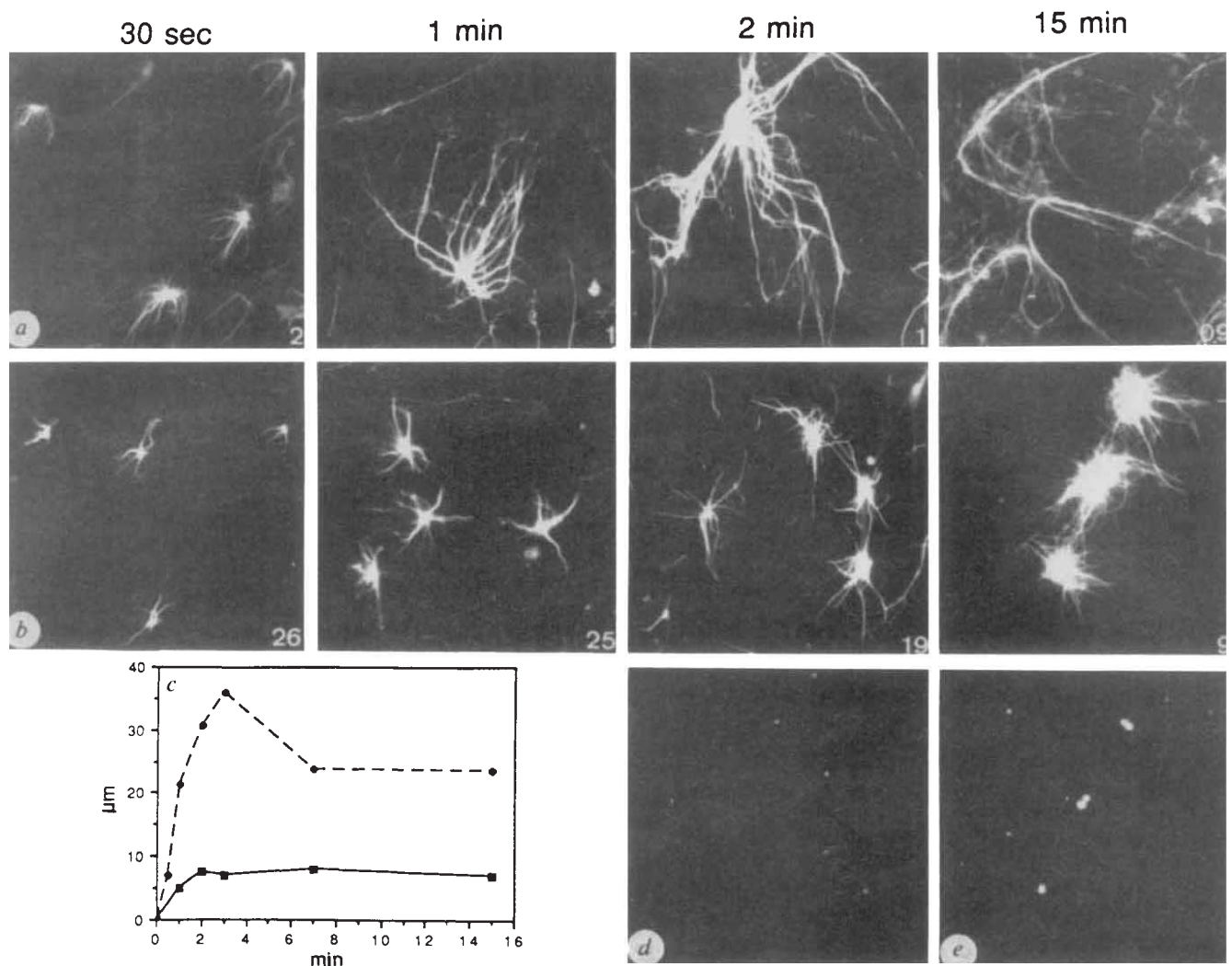


FIG. 4 Effect of *cdc2* kinase on the mean elongation rate of microtubules in interphase extracts. Kinetics of microtubule assembly after addition of centrosomes to interphase extracts pre-incubated for 15 min at room temperature without (a) and with (b) *cdc2* kinase. c, Quantitation of microtubule elongation in extracts pre-incubated without (dashed line) and with (solid line) *cdc2*. The length distribution gave an average dispersion of

$\pm 11 \mu\text{m}$ from the mean in the untreated extract and $\pm 3 \mu\text{m}$ in the *cdc2*-treated extract. d, e, MPM2 staining of the centrosomes that nucleate microtubules in the Figs above. The interphase extract used in this experiment was prepared from eggs activated¹⁴ and incubated in cycloheximide for 2.5 h as in all of the following figures. Magnification, $\times 825$.

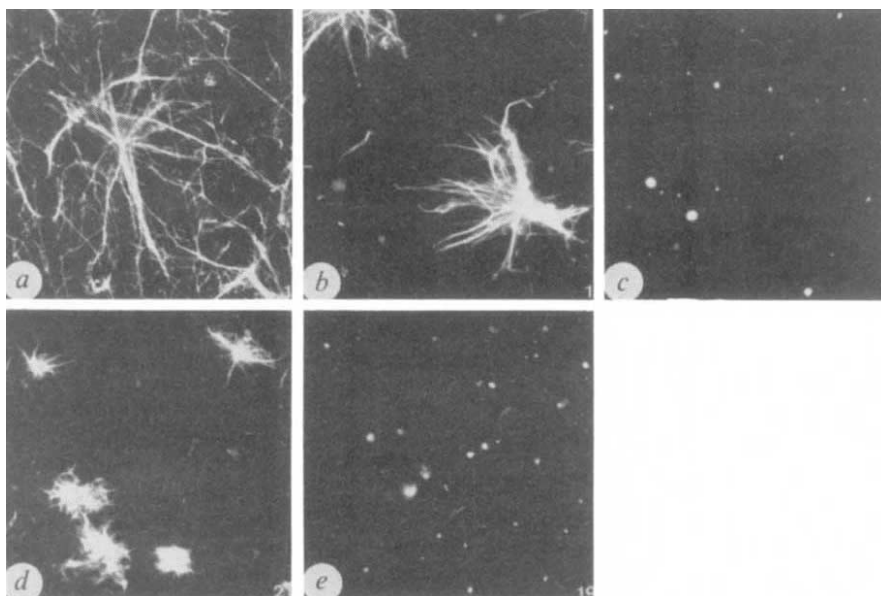


FIG. 5 Kinetics of microtubule depolymerization in response to nocodazole. Centrosomes were added to an interphase extract without (a-c) or with (d, e) *cdc2* and the reaction mixture incubated for 15 min at room temperature. Then nocodazole ($20 \mu\text{M}$) was added and aliquots fixed after 1 min (a), 10 min (b) and 20 min (c) for the interphase extract. Aliquots were fixed after 1 min (d) and 2.5 min (e) for the *cdc2*-treated extract. Other time points are not shown. H1 kinase activity is indicated in the lower right corner of each picture. Magnification, $\times 825$.

microtubules without spontaneous polymerization, followed by microtubule shrinking between 5 and 7 min after the beginning of the incubation. Microtubules reached the steady-state mitotic length at 15 min (Fig. 6a). Addition of 1 mM 6-DMAP (dimethyl amino purine), a nonspecific kinase inhibitor^{25,34,35}, to the extracts completely inhibited the microtubule shrinking induced by cdc2 kinase (Fig. 6b) and suppressed protein phosphorylation in the extract (Fig. 6c). In the absence of 6-DMAP, protein phosphorylation was significant after 7 min and maximal at 15 min. (Fig. 6c). This is in good agreement with the timing of microtubule shrinking. The kinase preparation that we used in this experiment had cdc2 and cyclin associated in a 1:1 complex²². When we added radioactive ATP to the pure preparation, the cyclin subunit was strongly phosphorylated, but this was not the case when we incubated the kinase in the extract (Fig. 6c). Only a few centrosomal proteins were weakly labelled by incubation of pure centrosomes with cdc2 kinase.

Mean microtubule length and cdc2 kinase activity

We verified that cdc2 kinase has no effect on pure tubulin polymerization from isolated centrosomes (data not shown). Therefore, a factor of the extract involved in the regulation of microtubule dynamics and length is phosphorylated. We were interested in determining whether microtubules shrink abruptly above a threshold concentration of cdc2 kinase concentration, or whether the mean length of microtubules is modulated as a continuous function of the kinase activity in the extract. The result for an extract is shown in Fig. 7. The mean steady-state

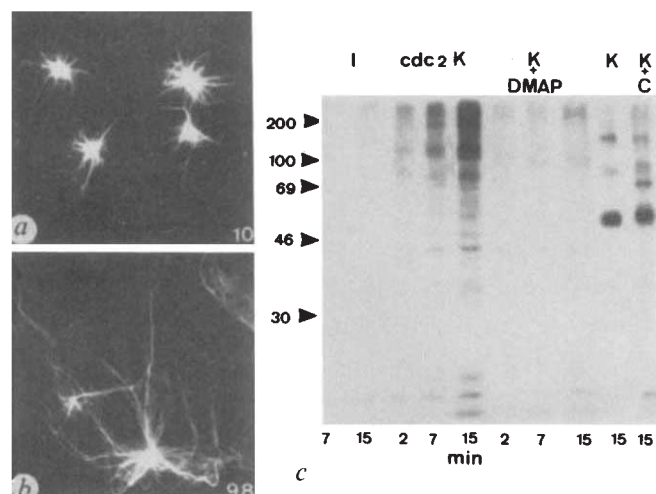


FIG. 6 Microtubule dynamics is regulated by phosphorylation. Centrosomes and cdc2 kinase were added together to an interphase extract in the absence (a) and in the presence (b) of 1 mM 6-DMAP. Samples were fixed after 15 min and processed for double immunofluorescence with a rabbit anti-tubulin antibody revealed by fluorescein-labelled goat anti-rabbit antibody and the MPM2 monoclonal antibody revealed by a rhodamin-conjugated goat anti-mouse antibody (results not shown). c. Patterns of protein phosphorylation. From the left: interphase extract labelled for 7 and 15 min with ^{32}P ; interphase extract containing cdc2 kinase ($20 \text{ pmol min}^{-1} \mu\text{l}^{-1}$) and labelled for 2, 7 and 15 min; interphase extract containing cdc2 kinase ($20 \text{ pmol min}^{-1} \mu\text{l}^{-1}$) and 1 mM 6-DMAP, labelled for 2, 7 and 15 min; autophosphorylation of the cdc2 kinase preparation; phosphorylation of purified centrosomes by cdc2 kinase. These last two experiments were carried out with the same amount of kinase as that added to the extracts, in a similar volume of acetate buffer containing 1 mM cold ATP. About 5×10^4 centrosomes were loaded on the gel. Phosphorylation was carried out for 15 min at room temperature.

METHODS. c. Aliquots of extracts (7 μl) were added to Eppendorf tubes containing 10 μCi dry [$\gamma\text{-}^{32}\text{P}$] ATP (Amersham, PB 218). After incubation, 40 μl stop buffer (50 mM NaF, 40 mM β-glycerophosphate, 10 mM EDTA, 10 mM sodium pyrophosphate, pH 7.2) were added to block kinases and phosphatases. Three volumes of SDS gel sample buffer were then added and the same number of free counts loaded on a 10% minigel according to Laemmli⁴⁸.

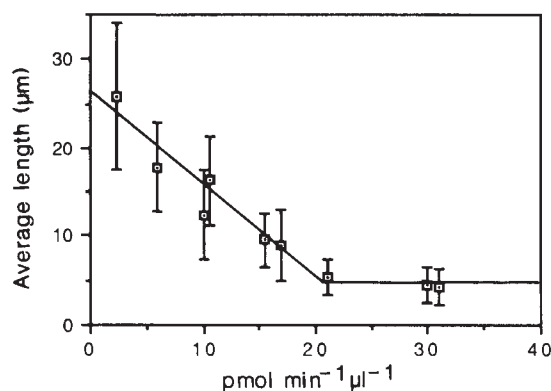


FIG. 7 Correlation between steady-state microtubule length and cdc2 kinase activity in the extracts. Centrosomes were added to an interphase extract with increasing amounts of cdc2 kinase. The samples were fixed after a 15-min incubation at room temperature. For each sample, the microtubule mean length was plotted versus the cdc2 kinase activity measured during the incubation (the kinase activity used is the average between the values at the beginning and end of the 15-min incubation at room temperature). The slope has a negative value of 0.99 and the correlation coefficient for linearity is 0.914. Bars indicate s.d. of microtubule length from the mean value.

microtubule length decreased linearly (correlation coefficient = 0.914) with increasing kinase activities between 2.5 and 22 pmol per min per μl extract. Above this level of kinase activity, the mean length of microtubules remained stable at ~4 μm.

This result indicates that a factor regulated by phosphorylation adjusts the steady-state microtubule length by modulating the dynamic properties of microtubules. The concentration of active factor is probably determined by the relative amount of kinase activity and the corresponding phosphatase. We are now investigating the nature of this phosphatase. It seems to be a type-1 phosphatase that counteracts the kinase and regulates microtubule dynamics and steady-state length. This is indicated by specific inhibitors of phosphatase 1 (inhibitors 1 and 2) strongly potentiating the effect of cdc2 kinase on microtubule dynamics (F.V. *et al.*, manuscript in preparation).

Discussion

We have shown here that in interphase *Xenopus* egg extracts, cdc2 kinase induces a change in microtubule dynamics similar to that observed *in vivo* during the interphase-metaphase transition. The phosphorylation of proteins in the extracts seems to be essential in this process. This is consistent with ATP being required for heightened microtubule turnover in mitosis^{36,37}. It is still unclear whether the cyclin subunit is required for the regulation of microtubule dynamics. Indeed, there are cyclin mutants in yeast (*cdc13-117*)^{38,39} in which cdc2 kinase is activated normally during mitosis and chromosomes condense, but interphasic microtubules do not shrink and the mitotic spindle does not assemble. This remains to be investigated in our system because cdc2 kinase apparently without the intact cyclin subunit²³ also induces mitotic microtubule dynamics.

The high microtubule elongation rate that we observed in interphase extracts ($20 \mu\text{m min}^{-1}$) was due to the presence of a specific microtubule-associated protein (X-MAP) that promotes microtubule elongation at the plus end¹⁶. It is possible that the reduced elongation rate ($4 \mu\text{m min}^{-1}$) and increased microtubule turnover that we observed in mitotic extracts were both due to inactivation of X-MAP by phosphorylation. But in somatic cells there is no X-MAP¹⁶, the microtubule elongation rates in interphase and during mitosis are very similar ($\sim 4 \mu\text{m min}^{-1}$; refs 40-42), and yet the turnover of microtubules is 10-fold higher during mitosis^{7,8}, as is the case in *Xenopus* eggs. X-MAP is believed to support the rapid growth of the sperm aster across the egg over almost 1 mm. Maybe its activity is cancelled during mitosis in the same time as the ubiquitous mitotic regime is established.

The finding that microtubules stop growing after they reach a short length in metaphase extracts or in interphase extracts treated with cdc2 kinase is interesting. With pure brain tubulin at $\sim 10 \mu\text{M}$, microtubules elongate at $\sim 1 \mu\text{m min}^{-1}$ from centrosomes or axonemes⁴³⁻⁴⁵. Yet these pure microtubules growing at slow rates can form asters with a mean microtubule length of $15 \mu\text{m}$ after 30 min of growth⁴³. But in metaphase extracts, microtubules growing at a much higher rate ($4 \mu\text{m min}^{-1}$) stop elongating after they reach mean lengths of 4 to $7 \mu\text{m}$. According to the dynamic instability model, some microtubules can grow while others shrink on the same centrosome⁴⁴. During mitosis, there is probably a combination of factors regulated by the cdc2 kinase that allows microtubules to grow quickly for a few minutes and then to shrink rapidly. This would produce asters of very dynamic microtubules with a specific steady-state mean

length. The actual rates of the addition and removal of tubulin subunits at microtubule ends, and the frequency of transition between growing and shrinking phases⁴⁵, should now be determined by video microscopy in interphasic and mitotic extracts. In any case, the determination of microtubule length by an enzymatic process modulating microtubule dynamic instability is of considerable general interest, and could be one of the key mechanisms determining the overall dimension of the mitotic spindle. Strikingly, the mean length of microtubules is directly correlated with the level of cdc2 kinase activity present in the extracts that we used, and *in vivo*, between prophase and metaphase, there is a precise temporal control of cdc2 kinase activation^{25,39}. It seems that the question of how spatio-temporal events are integrated in the cell through the regulation of cdc2 kinase activity is beginning to be unravelled. $\square$

Received 20 September; accepted 8 December 1989.

- Wilson, E. B. *The Sources of Science* 30, 45-87 (Johnson Reprint Corporation, 1966).
- Inoue, S. *J. Cell Biol.* **51**, 1315-1475 (1981).
- Vorobjev, I. A. & Chentsov Yu. S. *J. Cell Biol.* **98**, 938-949 (1982).
- Kuriyama, R. & Borisy, G. G. *J. Cell Biol.* **91**, 822-826 (1981).
- Bajer, A. & Mole-Bajer, J. *Chromosoma* **27**, 448-484 (1969).
- Vandre, D. D., Kronebush, P. & Borisy, G. In *Molecular Biology of the Cytoskeleton* (eds Borisy, G. G., Cleveland, D. W. & Murphy, D. B.) 3-16 (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1984).
- Salmon, E. D., Saxton, W. M., Leslie, R. J., Karow, M. L. & McIntosh, R. J. *J. Cell Biol.* **99**, 2157-2164 (1988).
- Saxton, W. M. *et al. J. Cell Biol.* **99**, 2175-2186 (1984).
- Nicklas, R. B. *Adv. Cell Biol.* **2**, 225-297 (1971).
- Rieder, C. L. *Int. Rev. Cytol.* **79**, 1-57 (1982).
- Mitchison, T. J. *A. Rev. Cell Biol.* **4**, 527-549 (1988).
- McIntosh, J. R. *J. Cell Biol.* **83**, 428-442 (1979).
- Kirschner, M. & Mitchison, T. *Cell* **45**, 329-342 (1986).
- Karsenti, E., Newport, J., Hubble, R. & Kirschner, M. *J. Cell Biol.* **98**, 1730-1745 (1984).
- Gard, D. L. & Kirschner, M. W. *J. Cell Biol.* **105**, 2191-2201 (1987).
- Gard, D. L. & Kirschner, M. W. *J. Cell Biol.* **105**, 2203-2215 (1987).
- Manes, M. & Barbieri, F. *J. embryol. exp. Morph.* **40**, 187-197 (1977).
- Stewart-Savage, J. & Grey, R. D. *Wilhelm Roux Arch. dev. Biol.* **191**, 241-245 (1982).
- Lohka, M. & Maller, J. *J. Cell Biol.* **101**, 518-523 (1985).
- Lhoka, M. F., Hayes, M. K. & Maller, J. L. *Proc. natn. Acad. Sci. U.S.A.* **85**, 3009-3013 (1988).
- Labbé, J. C. *et al. Cell* **57**, 253-263 (1989).
- Labbé, J. C. *et al. EMBO J.* **8**, 3053-3058 (1989).
- Labbé, J. C., Lee, M. G., Nurse, P., Picard, A. & Doree, M. *Nature* **335**, 251-254 (1988).
- Doree, M., Labbé, J. C. & Picard, A. *J. Cell Sci.* (in the press).
- Felix, M. A., Pines, J., Hunt, T. & Karsenti, E. *EMBO J.* **8**, 3059-3069 (1989).

- Murray, A. & Kirschner, M. *Nature* **339**, 275-280 (1989).
- Minshull, J., Blow, J. & Hunt, T. *Cell* **56**, 947-956 (1989).
- Gould, K. L. & Nurse, P. *Nature* **342**, 39-45 (1989).
- Gautier, J., Matsukawa, T., Nurse, P. & Maller, J. *Nature* **339**, 626-629 (1989).
- Capon, J. P., Picard, A., Peaucellier, G., Labbé, J. C. & Doree, M. *Dev. Biol.* **117**, 1-12 (1986).
- Vandre, D. D., Davis, F. M., Rao, P. T. & Borisy, G. G. *Proc. natn. Acad. Sci. U.S.A.* **81**, 4439-4443 (1984).
- Vandre, D. D., Davis, F. M., Rao, P. T. & Borisy, G. G. *Eur. J. Cell Biol.* **41**, 72-81 (1986).
- DeBrabander, M. *et al. Int. Rev. Cytol.* **101**, 215-274 (1986).
- Neant, I. & Guerrier, P. *Exp. Cell Res.* **176**, 68-79 (1988).
- Neant, I., Charbonneau, M. & Guerrier, P. *Dev. Biol.* **132**, 304-314 (1989).
- DeBrabander, M., Guens, G., Nuydens, R., Willebrords, R. & DeMey, J. *Cell Biol. int. Rep.* **5**, 913-920 (1981).
- Wadsworth, P. & Salmon, E. D. *Cell Motil.* **11**, 97-105 (1988).
- Hagan, I. M., Hayles, J. & Nurse, P. J. *Cell Sci.* **91**, 587-595 (1988).
- Moreno, S., Hayles, J. & Nurse, P. *Cell* **58**, 361-372 (1989).
- Schulze, E. & Kirschner, M. *J. Cell Biol.* **102**, 1020-1031 (1986).
- Sammak, P. J. & Borisy, G. G. *Nature* **332**, 724-726 (1988).
- Mitchison, T., Evans, L., Schulze, E. & Kirschner, M. *Cell* **45**, 515-527 (1986).
- Bre, M. H. & Karsenti, E. *Cell Motil.* (in the press).
- Mitchison, T. & Kirschner, M. *Nature* **312**, 232-237 (1984).
- Walker, R. A. *et al. J. Cell Biol.* **107**, 1437-1448 (1988).
- Bornens, M., Paintrand, M., Berges, J., Marty, M. C. & Karsenti, E. *Cell Motil.* **8**, 238-249 (1987).
- Evans, L., Mitchison, T. & Kirschner, M. *J. Cell Biol.* **100**, 1185-1191 (1985).
- Laemli, U. K. *Nature* **227**, 680-685 (1970).

ACKNOWLEDGMENTS. We acknowledge Jan DeMey and Marie-Anne Felix for discussions and suggesting key experiments. We also thank J. Kenney, K. Simmons and J. Tooze for critical reading of the manuscript, B. Maier and A. Summerfield for artwork, and A. Walter for secretarial assistance.

LETTERS TO NATURE

Unusually low heavy-element abundance found in the blue compact dwarf galaxy SBS0335-052

Yu. I. Izotov*, V. A. Lipovetskii†, N. G. Guseva*, A. Yu. Kniazev† & J. A. Stepanian‡

* Main Astronomical Observatory, Ukrainian Academy of Sciences, Goloseevo, 252127, USSR

† Special Astrophysical Observatory, USSR Academy of Sciences, 357147, Nizhny Arhyz, Kiev, USSR

‡ Byurakan Astrophysics Observatory, Armenian Academy of Sciences, 378433, Byurakan, USSR

BLUE compact dwarf galaxies are noted for their high star-formation activity and young age. They can therefore be used to verify models of galaxy formation, the chemical evolution of matter and the evolution of massive stars, for example. Here we report observations of the blue compact dwarf galaxy, SBS0335-052, which show that this galaxy has an extremely low heavy-element abundance; the oxygen abundance is 77-times lower than the solar value and 1.7-times lower than that found in another galaxy, I Zw 18, which was the most deficient in heavy elements. The electron temperature, 24,800 K, is very high which leads us to conclude

that there are a significant number of stars with masses $\approx 100 M_{\odot}$ and effective temperatures of ionizing stars of up to 8×10^4 K in the galaxy. Our observations imply that SBS0335-052 is very young.

The evidence for the young age of blue compact dwarf galaxies includes their significant enrichment with gas (10-75% of the total galaxy mass), their heavy element deficiency (1/2-1/40 of the Sun's heavy-element abundance with a mean value of 1/10, see refs 1-3) and the lack of the late-star population in some cases (refs 4,5). The first burst of star formation may be occurring in some of these galaxies.

We observed⁶ the blue compact dwarf galaxy SBS0335-052, (found in the Second Byurakan Survey) for the first time in November 1988 using the 1,000-channel spectrophotometer of the 6-m telescope of the Special Astrophysical Observatory of the Soviet Academy of Sciences. We found that this galaxy had an exceptionally low abundance of heavy elements relative to other blue compact dwarf galaxies. The right ascension (1950) and declination (1950) of SBS0335-052 are $03^{\text{h}}35^{\text{m}}18^{\text{s}}$ and $-05^{\circ}12'34''$, and the distance d is $(73.7 \pm 1.5)/h_{50}$ Mpc, where h_{50} is $H/50$ and H is the Hubble constant. The absolute photographic magnitude is $M = -16.3$. On 25 January 1989 we carried out spectrophotometric observations of SBS0335-052 using the echelle spectrograph of the 6-m telescope with high signal-to-noise ratio. We show the uncorrected spectrum of SBS0335-052 in Fig. 1. We present the emission line intensities, corrected for underlying stellar absorption (ref. 7) and extinction (ref. 8), in

TABLE 1 Line intensities in spectrum of SBS0335-052

No.	λ_0 Å	Ion	Extinction factor f	Intensities	
				observed	corrected
1	3,726	[O II]	0.315	0.073 ± 0.006	0.076 ± 0.006
2	3,729	[O II]	0.315	0.064 ± 0.005	0.067 ± 0.005
3	3,750	H ₁₂	0.308	0.020 ± 0.002	0.034 ± 0.003
4	3,771	H ₁₁	0.301	0.018 ± 0.002	0.030 ± 0.003
5	3,798	H ₁₀	0.290	0.029 ± 0.003	0.044 ± 0.003
6	3,835	H ₉	0.280	0.058 ± 0.005	0.075 ± 0.006
7	3,869	[Ne III]	0.279	0.168 ± 0.010	0.174 ± 0.010
8	3,889	He I + H ₈	0.265	0.136 ± 0.009	0.141 ± 0.009
9	3,969	[Ne III] + He	0.235	0.157 ± 0.010	0.162 ± 0.010
10	4,102	H ₈	0.200	0.241 ± 0.014	0.263 ± 0.015
11	4,340	H _γ	0.135	0.454 ± 0.023	0.476 ± 0.024
12	4,363	[O III]	0.130	0.120 ± 0.008	0.122 ± 0.008
13	4,472	He I	0.105	0.048 ± 0.004	0.049 ± 0.004
14	4,686	He II	0.045	0.020 ± 0.002	0.020 ± 0.002
15	4,712	[Ar IV] + He I	0.035	0.017 ± 0.002	0.017 ± 0.002
16	4,717	[Ne IV]	0.033	0.008 ± 0.001	0.008 ± 0.001
17	4,740	[Ar IV]	0.025	0.011 ± 0.001	0.011 ± 0.001
18	4,861	H _β	0.000	1.000 ± 0.024	1.000 ± 0.024
19	4,959	[O III]	-0.020	0.902 ± 0.041	0.900 ± 0.041
20	5,007	[O III]	-0.030	2.603 ± 0.102	2.592 ± 0.102
21	5,876	He I	-0.210	0.165 ± 0.012	0.160 ± 0.012
22	6,563	H _α	-0.335	2.872 ± 0.116	2.748 ± 0.111
23	6,584	[N II]	-0.340	<0.008 ± 0.001	<0.008 ± 0.001
24	6,678	He I	-0.360	0.041 ± 0.005	0.039 ± 0.005
25	6,717	[S II]	-0.370	0.025 ± 0.003	0.024 ± 0.003
26	6,731	[S II]	-0.370	0.024 ± 0.004	0.023 ± 0.004
27	7,006	[Ar V]	-0.392	0.043 ± 0.008	0.041 ± 0.008
EW(H _β), Å				175 ± 5	
$c_{H\beta}$				0.06	

Table 1, where EW (H_β) and $c_{H\beta}$ are the H_β equivalent width and galactic extinction, respectively.

The electron temperature of the ionized gas, which we determined from the line-intensity ratio ([O III] λ = 4,959 + 5,007 Å)/[O III] λ = 4,363 Å (ref. 9) is extremely high T_e = 24,800 ± 1,990 K. We determined the electron number density, N_e = 250 ± 150 cm⁻³, of ionized gas from the ratio of [S II] λ = 6,717 and λ = 6,731 Å.

We calculated the gas ion concentrations of the galaxy using the formulae in ref. 9. We have corrected the helium and heavy-element abundances using the correction factors given in refs 10, 11 which are based on model calculations of the H II region (refs 12-14).

$$\frac{O}{H} = \frac{O^+ + O^{2+}}{H^+}$$

$$\frac{Ne}{H} = \frac{Ne^{2+} + Ne^{3+}}{H^+}$$

$$\frac{S}{H} = \frac{1}{4} \frac{S^+}{H^+} \frac{O}{O^+}$$

$$\frac{N}{H} = \frac{N^+}{H^+} \frac{O}{O^+}$$

$$\frac{Ar}{H} = \frac{Ar^{3+} + Ar^{4+}}{H^+} \frac{He}{He^+}$$

$$\frac{He}{H} = \frac{He^+}{H^+} \frac{1}{1 - 0.25(O^+/O)}$$

The ion abundances and chemical composition of SBS0335-052 are given in Table 2. I Zw 18 and GR8 are well known to be the most heavy-element-deficient galaxies, having oxygen abundances ~1/40 (ref. 15) and ~1/30 (ref. 16) of the solar value, respectively. The oxygen abundance in SBS0335-052 is only 1/77 of the solar value, considerably lower than that in I Zw 18 and GR8. Hence the galaxy SBS0335-052 is the most heavy-element-deficient blue compact dwarf galaxy that has been observed so far. We therefore conclude that SBS0335-052 is probably one of the youngest galaxies, that its matter is of primaeval chemical composition and that its first burst of the star formation is occurring.

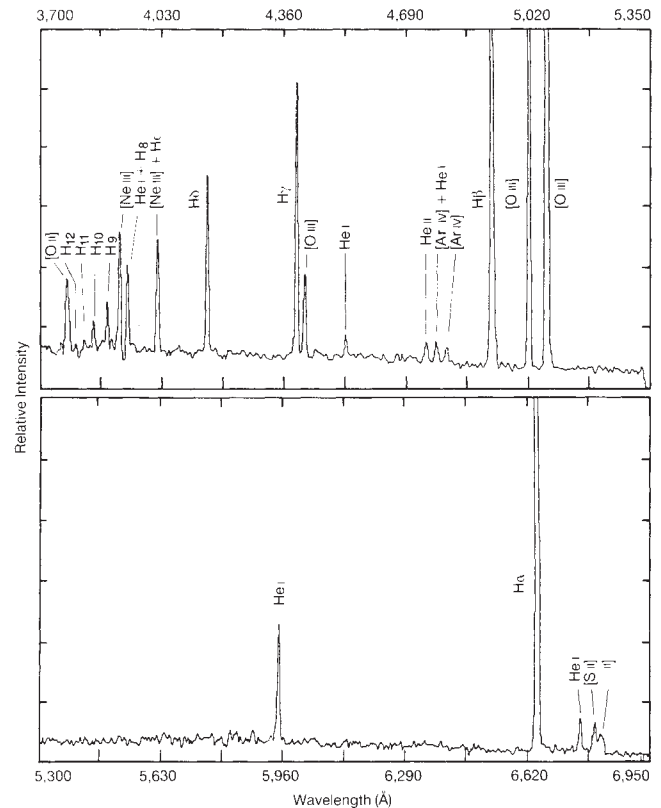


FIG. 1 The uncorrected spectrum of the galaxy SBS0335-052. Observation time is 5,400 s on 25 January 1989.

Unfortunately our observations lack absolute calibration. Therefore we have estimated the effective temperature of ionizing stars $T_* \approx 8 \times 10^4$ K, the ionization parameter $U \approx 0.2$ and filling factor $f = 1$ by extrapolating the results of model calculations¹⁷. The ionization temperature T_* is exceptionally high and can be satisfactorily explained by the evolution of massive stars that had initial masses $M > 40 M_\odot$ and which are effectively losing their masses¹⁸.

It is common practice to use the equivalent width of the hydrogen line H_β to determine the age of H II regions. The age of the H II region in SBS0335-052 determined in this way is

TABLE 2 Ionic and chemical composition of the gas in SBS0335-052

	SBS0335-052	I Zw 18		Sun
		ref. 15	ref. 20	
T_e (K)	24,800 ± 1,990			
N_e (cm ⁻³)	250 ± 150			
O^+/H^+	(3.59 ± 1.15) × 10 ⁻⁷	1.70 × 10 ⁻⁶		
O^{2+}/H^+	(9.89 ± 2.97) × 10 ⁻⁶	1.35 × 10 ⁻⁵		
Ne^{2+}/H^+	(1.30 ± 0.40) × 10 ⁻⁶	2.75 × 10 ⁻⁶		
Ne^{3+}/H^+	(2.21 ± 1.21) × 10 ⁻⁶			
Ar^{3+}/H^+	(5.40 ± 1.82) × 10 ⁻⁸			
Ar^{4+}/H^+	(7.95 ± 2.91) × 10 ⁻⁸			
S^+/H^+	(1.84 ± 0.90) × 10 ⁻⁸	6.31 × 10 ⁻⁸		
N^+/H^+	< (2.67 ± 0.98) × 10 ⁻⁷			
He^+/H^+ (4,472 Å)	0.113 ± 0.011			
He^+/H^+ (5,876 Å)	0.145 ± 0.014			
He^+/H^+ (6,678 Å)	0.124 ± 0.018			
He^+/H^+ (average)	0.127 ± 0.014	0.074		
He^{2+}/H^+ (4,686 Å)	(2.14 ± 0.27) × 10 ⁻³	< 3.10 ⁻³		
12 + log(He/H)	11.10 ± 0.05	10.88		11.00
12 + log(O/H)	7.01 ± 0.14	7.18	7.24 ± 0.07	8.90
12 + log(Ne/H)	6.55 ± 0.20	6.49	6.56 ± 0.09	8.00
12 + log(Ar/H)	5.13 ± 0.21	—	—	6.58
12 + log(S/H)	5.12 ± 0.48	—	5.46 ± 0.30	7.21
12 + log(N/H)	< 5.88 ± 0.45	—	5.99 ± 0.16	7.95

3.3×10^6 yr. We believe that the high value of T_* and the young age of H II region in SBS0335–052 can be explained by the radiation of a moderate number of stars with initial masses of $\sim 100 M_\odot$ that are now in the Wolf–Rayet stage. Because of the absence of Wolf–Rayet spectral features near the line He II $\lambda = 4,686 \text{ \AA}$, the number of Wolf–Rayet stars is probably only a few per cent of total star number. The estimated flux of Lyman continuum radiation is $N_{\text{Ly}\alpha} = 2 \times 10^{53} \text{ s}^{-1}$, which corresponds to the radiation of a few hundred stars with the masses we require.

In massive stars that lose mass before reaching the Wolf–Rayet stage the gas is substantially enriched in helium. The helium mass fraction in matter with primaeval chemical composition is usually estimated to be $Y = 0.23\text{--}0.25$. Then the extra mass fraction ΔY of He at age $\approx 3.3 \times 10^6$ yr is 0.07 and the value of $12 + \log(\text{He}/\text{H})$ is 11.07, if we take into account the evolution

of all stars with masses greater than $50 M_\odot$. This value of $12 + \log(\text{He}/\text{H})$ is commonly observed in blue compact dwarf galaxies.

The observed abundance of oxygen and neon can be explained by mass loss in the estimated number of stars with the highest mass $\approx 10^2 M_\odot$ (ref. 19), if the mass in the H II region has the usual value $\approx 10^6 M_\odot$. In this case the mass ratio Ne/O is ~ 0.42 , which is in satisfactory agreement with observed value $(\text{Ne}^{2+} + \text{Ne}^{3+})/(\text{O}^+ + \text{O}^{2+}) \approx 0.55$.

To confirm that SBS0335–052 is a young galaxy in which the first burst of star formation is occurring optical spectrophotometric observations with absolute calibration and observations in the near infrared range to search for a late star population are required. Ultraviolet observations of SBS0335–052 are also necessary to study the most massive stars in the galaxy. $\square$

Received 12 July; accepted 9 November 1989.

- Dufour, R. J. *Publ. astr. Soc. Pacif.* **98**, 1025–1031 (1986).
- Kunth, D. *Sci. Rep. Tohoku Univ. 8th Ser.*, **7**, 353–363 (1987).
- Kunth, D. & Sargent, W. L. W. *Astrophys. J.* **300**, 496–499 (1986).
- Kunth, D., Martin, J. M., Maurogordato, S. & Vigroux, L. *Astr. Astrophys.* **204**, 10–20 (1988).
- Loose, H. H. & Thuan, T. X. T. *Proc. Workshop Star-forming Dwarf Galaxies and Related Objects* 73–88 (Editions Frontières, Gif sur Yvette, 1986).
- Izotov, Yu. I., Lipovetskii, V. A., Guseva, N. G. & Stepanian, J. A. *Special Astrophysical Observatory Preprint No. 30*, Academy of Sciences of USSR (1989).
- McCall, M. L., Rybski, P. M. & Shields, G. A. *Astrophys. J. Suppl. Ser.* **57**, 1–62 (1985).
- Whitford, A. E. *Astr. J.* **63**, 201–206 (1958).
- Aller, L. H. *Physics of Thermal Gaseous Nebulae* (Reidel, Dordrecht, 1984).

- Perrinotto, M. in *Diffuse Matter in Galaxies* (Reidel, Dordrecht, 1983).
- Gonzales-Riestra, R., Rego, M. & Zamorano, J. *Astr. Astrophys.* **202**, 27–34 (1988).
- Stasinska, G. *Astr. Astrophys.* **66**, 257–267 (1978).
- Stasinska, G. *Astr. Astrophys.* **84**, 320–328 (1980).
- Stasinska, G. *Astr. Astrophys. Suppl.* **48**, 299–304 (1982).
- Lequeux, J., Peimbert, M., Rego, J. F., Serrano, A. & Torres-Peimbert, S. *Astr. Astrophys.* **80**, 155–160 (1979).
- Skillman, E. D., Melnick, J., Terlevich, R. & Moles, M. *Astr. Astrophys.* **196**, 31–38 (1988).
- Campbell, A. *Astrophys. J.* **335**, 644–657 (1988).
- Maeder, A. & Meynet, G. *Astr. Astrophys.* **182**, 243–263 (1987).
- Maeder, A. *Astr. Astrophys.* **120**, 113–129 (1983).
- Dufour, R. J., Garnett, D. R. & Shields, G. A. *Astrophys. J.* **332**, 752–762 (1988).
- Grevesse, N. *Physica scripta* **8**, 49–58 (1984).

The parallax and proper motion of PSR1451–68

M. Bailes*, R. N. Manchester†, M. J. Kesteven†, R. P. Norris† & J. E. Reynolds*

* Mount Stromlo and Siding Spring Observatories, Institute of Advanced Studies, Australian National University, Private Bag, Woden PO, ACT 2606, Australia

† Australia Telescope National Facility, CSIRO, PO Box 76, Epping, NSW 2121, Australia

MEASUREMENTS of annual parallax give distances for stellar objects which are largely model-independent. Such measurements provide a basis for the calibration of other methods for estimating distances which are applicable to more distant objects. In the case of pulsars, parallax measurements allow estimation of the local interstellar electron density and hence calibration of the distance scale based on pulse dispersion. Here we use data from the Parkes–Tidbinbilla interferometer to determine the parallax and proper motion of PSR1451–68, obtaining a value of 2.2 ± 0.3 milliarcseconds for the parallax and 41.4 ± 0.9 milliarcseconds per year for the proper motion. When combined with the dispersion measure, the parallax yields a value of $0.019 \pm 0.003 \text{ cm}^{-3}$ for the mean density of free electrons along the line of sight to this pulsar. Together with other measurements, this result indicates that the interstellar medium within 500 pc of the Sun is typical of that at greater distances.

As part of a survey to determine the proper motions of several radio pulsars visible in the Southern Hemisphere using the Parkes–Tidbinbilla interferometer (PTI)^{1,2}, we observed the pulsar PSR1451–68 together with a nearby reference source on several occasions between November 1986 and November 1988. The PTI is a radio-linked interferometer with a baseline of 275 km and at the observing frequency of 1.66 GHz has a minimum fringe spacing of 135 mas. An unresolved reference source with a flux density of $\sim 100 \text{ mJy}$ was found only ~ 6 arcmin from the pulsar. This pulsar is strong (mean flux density $\sim 70 \text{ mJy}$) and is relatively close to the Sun (dispersion measure of only $8.6 \text{ cm}^{-3} \text{ pc}$) making it an excellent candidate for the determination of a trigonometric parallax.

We made observations in nine sessions (see Fig. 1 legend), covering a wide range of hour angles. Both the pulsar and the reference source were within the main beam of the interferometer allowing precise measurements of the differential interferometric phase. We calculated interferometric phases relative to a phase centre at an assumed position separately for the pulsar and the reference source. We then calculated the differences between the observed phases for these two positions as a function of time. An error in the assumed relative position of the pulsar and reference source results in variations of the phase difference with hour angle, whereas changes in the relative position with epoch of observation result in variations of the phase difference at a given hour angle. We have fitted a model for the variations in the phase differences resulting from proper motion and parallax of the pulsar to the data. We assumed that the reference source is extragalactic and hence has zero proper motion (in the extragalactic reference frame) and zero parallax. Figure 1 shows the phase differences averaged over five-minute intervals at each of the epochs of observation, and the best-fit proper motion and parallax solution. If the parallax were zero, the phase curves for all epochs would have a common point of intersection. Hence the displacements of the best-fit curves from the intersection point near zero hour angle illustrate the significance of the parallax.

We obtained the solution from a standard least-squares regression in which the weight of each point is inversely proportional to the r.m.s. deviation in the five-minute average. The resulting values for the proper motion are $\mu_\alpha = -39.5 \pm 0.7 \text{ mas yr}^{-1}$ and $\mu_\delta = -12.3 \pm 0.6 \text{ mas yr}^{-1}$, where μ_α and μ_δ are the components of proper motion in the right ascension and declination directions respectively, and the annual parallax π is $2.2 \pm 0.3 \text{ mas}$. The r.m.s. scatter about the best-fit solution is only four degrees and the quoted errors are twice those given by the least-squares solution. Assumed positions (J2000 equinox) of the pulsar and reference source respectively were $\alpha = 14^{\text{h}}55^{\text{m}}58.0448^{\text{s}}$, $\delta = -68^{\circ}43'37.5393''$ and $\alpha = 14^{\text{h}}55^{\text{m}}19.2126^{\text{s}}$, $\delta = -68^{\circ}39'12.1894''$.

For a distance of 450 pc (see below), the proper motion of PSR1451–68 implies a transverse velocity of $88 \pm 2 \text{ km s}^{-1}$. The median velocity of the other pulsars for which velocities have been measured^{2,3} is $\sim 100 \text{ km s}^{-1}$. In galactic coordinates, the proper-motion vector has components of $-30.7 \text{ mas yr}^{-1}$ and

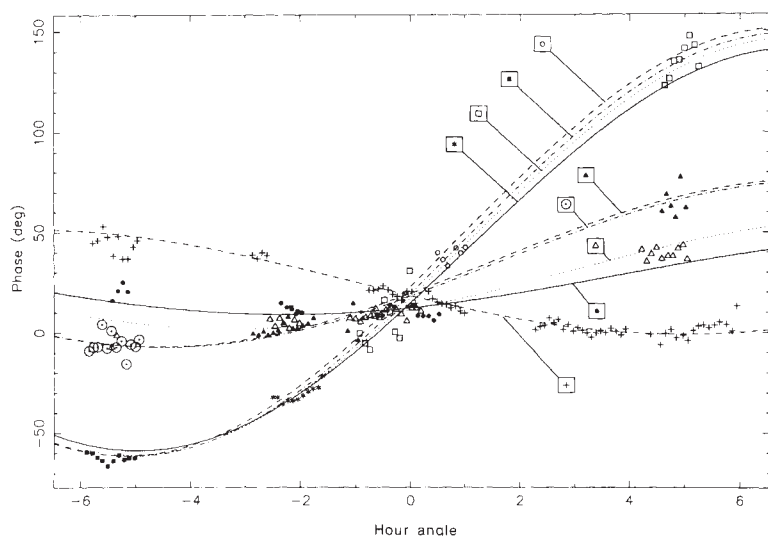


FIG. 1 The best-fit proper motion and parallax solution to the phase difference between the pulsar PSR1451-68 and a nearby reference source. Each symbol represents the phase at a different epoch and the curves represent the expected phase at each of the epochs. The proper motion is 41.4 ± 0.9 mas yr $^{-1}$. The observation sessions and symbols are: $\circ$, 9 November 1986; $\blacksquare$, 14 December 1986; $\square$, 4 January 1987; $*$, 29 January 1987; $\blacktriangle$, 28 November 1987; $\odot$, 11 December 1987; $\triangle$, 19 March 1988; $+$, 20 April 1988; $+$, 25 November 1988.

10.3 mas yr $^{-1}$ in longitude and latitude respectively. As the pulsar is at a galactic latitude of -8.5° , its motion is directed toward the galactic plane, unlike most other pulsars³. The pulsar has a large characteristic age, $\sim 4 \times 10^7$ yr, so it may have completed a half-cycle of an oscillation in the galactic potential, or alternatively it may have been born at a large distance from the galactic plane. (The present distance of the pulsar from the plane is ~ 65 pc.) If the pulsar has completed a half-cycle oscillation in the galactic potential, its actual age must be comparable to its characteristic age. This implies that decay of the magnetic field in this pulsar must have a very long (perhaps infinite) timescale. Nondecaying or very slowly decaying fields seem to be required in millisecond pulsars⁴. These pulsars are believed to be 'recycled', that is, to be spun up by momentum transfer in a binary system⁵ and it is possible that PSR1451-68 and pulsars like it have a similar evolutionary history.

The measured parallax for PSR1451-68 is the most accurate known for any pulsar. (Indeed, we believe that it is the most accurate trigonometric parallax ever measured.) Parallaxes have been reliably measured for only two other pulsars. Using the techniques of very-long-baseline interferometry, Gwinn *et al.*⁶ have determined the parallaxes of PSR0823+26 ($\pi = 2.8 \pm 0.6$ mas) and PSR0950+08 ($\pi = 7.9 \pm 0.8$ mas). Salter *et al.*⁷ obtained a parallax of 21.5 ± 8.0 mas for PSR1929+10 but for the same object Backer and Sramek⁸ placed an upper limit on the parallax of only 4 mas. Using pulse-timing observations, Rawley *et al.*⁹ have put an upper limit of 0.4 mas on the parallax of the millisecond pulsar PSR1937+21. It is unlikely, however, that many parallaxes will be determined using this technique as it is only applicable to pulsars having very small period irregularities and most such pulsars are relatively distant. The accuracy of our result is due primarily to the fact that we were fortunate enough to find a strong, unresolved reference source in the same primary beam as a strong pulsar. Another significant factor was the high observing frequency of the PTI. Together, these reduced the effects of perturbations such as ionosphere irregularities which can result in large systematic errors. Specifically, there is no evidence for significant diurnal variations in the phase beyond those expected from the geometric model. We therefore believe that annual phase variations due to ionospheric or possibly other perturbations are not likely to be important and hence that the parallax measurement is not significantly affected by systematic errors.

The derived parallax for PSR1451-68 implies a distance of 450 ± 60 pc. Together with the dispersion measure, this gives a value for the mean free-electron density along the path of 0.019 ± 0.003 cm $^{-3}$. For PSR0823+26, at a distance of ~ 360 pc,

the derived mean density is 0.055 ± 0.012 cm $^{-3}$, whereas for PSR0950+08 the corresponding values are ~ 130 pc and 0.023 ± 0.002 cm $^{-3}$. For a sample of nine pulsars, most of which are within 500 pc of the Sun, Salter *et al.*⁷ find a mean electron density of 0.029 ± 0.014 cm $^{-3}$. For the Salter *et al.* sample and for PSR0950+08, the derived mean electron densities are close to those obtained from the electron-density model of Lyne *et al.*¹⁰, but for PSR1451-68, the derived value is about half the model value and for PSR0823+26 it is about 25% larger than the model density. The Lyne *et al.* model is based principally on pulsar distance measurements obtained from observations of neutral-hydrogen absorption and a model for differential galactic rotation. The pulsars used to define the model have a median distance of ~ 2.5 kpc with many much more distant. The parallax results therefore indicate that, although there are considerable variations along individual lines of sight, the interstellar electron density within ~ 500 pc of the Sun is quite representative of the wider galactic interstellar medium. This result is not inconsistent with the deduction (see, for example, ref. 11) based on observations of soft X-ray emission^{12,13} that, within ~ 100 pc of the Sun, the interstellar medium is dominated by a hot tenuous gas having a mean electron density of only 0.004 cm $^{-3}$. It can be convincingly argued^{14,15} that most of the pulsar dispersion arises in denser regions of fully ionized gas which are also responsible for the observed diffuse H α emission. These regions occupy only a relatively small proportion of the total volume and may coexist with the hot X-ray-emitting gas. $\square$

Received 23 August; accepted 28 November 1989.

- Norris, R. P., Kesteven, M. J., Wellington, K. J. & Batty, M. J. *Astrophys. J. Suppl.* **67**, 85-91 (1988).
- Bailes, M., Manchester, R. N., Kesteven, M. J., Norris, R. P. & Reynolds, J. E. *Astrophys. J.* **343**, L53-L55 (1989).
- Lyne, A. G., Anderson, B. & Salter, M. J. *Mon. Not. R. astr. Soc.* **201**, 503-520 (1982).
- Kulkarni, S. R. *Astrophys. J.* **306**, L85-L89 (1986).
- van den Heuvel, E. P. J. *J. Astr. Astrophys.* **5**, 209-233 (1984).
- Gwinn, C. R., Taylor, J. H., Weisberg, J. M. & Rawley, L. A. *Astr. J.* **91**, 338-342 (1986).
- Salter, M. J., Lyne, A. G. & Anderson, B. *Nature* **280**, 477-478 (1979).
- Backer, D. C. & Sramek, R. A. *Astrophys. J.* **260**, 512-519 (1982).
- Rawley, L. A., Taylor, J. H. & Davis, M. M. *Astrophys. J.* **326**, 947-953 (1988).
- Lyne, A. G., Manchester, R. N. & Taylor, J. H. *Mon. Not. R. astr. Soc.* **213**, 613-639 (1985).
- Cox, D. P. & Reynolds, R. J. *A. Rev. Astr. Astrophys.* **25**, 303-344 (1987).
- Marshall, F. J. & Clark, G. W. *Astrophys. J.* **217**, L87-L91 (1977).
- Sanders, W. T., Kraushar, W. L., Nousek, J. A. & Fried, P. M. *Astrophys. J.* **287**, 633-652 (1984).
- Reynolds, R. J. *Astrophys. J.* **282**, 191-196 (1984).
- Kulkarni, S. & Heiles, C. in *Interstellar Processes* (eds Hollenbach, D. J. & Thronson, H. A.) 87-122 (Reidel, Dordrecht, 1987).

ACKNOWLEDGEMENTS. The Australian Telescope National Facility is operated in association with the Division of Radiophysics by CSIRO.

Absence of strong coupling in $\text{YBa}_2\text{Cu}_3\text{O}_7$ inferred from infrared conductivity

Z. Schlesinger*, R. T. Collins*, F. Holtzberg*, C. Feild*, N. E. Bickers† & D. J. Scalapino‡

* IBM T. J. Watson Research Center, Yorktown Heights, New York 10598, USA

† University of Southern California, Los Angeles, California 90089, USA

‡ University of California, Santa Barbara, California 93106, USA

THE mechanism of superconductivity in the recently discovered layered copper oxide compounds¹ is still unknown. In the conventional theory, electron pairing mediated by the exchange of low-energy bosons leads to superconductivity. If the boson energies are not too large compared to the energy gap Δ (that is, $\hbar\omega_0 \lesssim 10\Delta$), then the interaction of the electron-pair quasiparticles with these bosons leads to an enhancement of the ratio $2\Delta/kT_c$ (where T_c is the superconducting transition temperature), as well as to modifications to the spectrum of excitations above the energy gap. The term 'strong coupling' is used to denote these phenomena^{2,3}. Infrared spectroscopy can be used to probe these modifications, and thus to measure the spectrum of mediating excitations in strongly coupled superconductors. Here we report infrared conductivity data for $\text{YBa}_2\text{Cu}_3\text{O}_7$, and show that even up to energies of ≥ 0.3 eV there is no evidence for a pair-mediating retarded interaction of significant strength. These results suggest that a conventional picture of superconductivity, involving mediation by the exchange of bosons of energy much less than the Fermi energy ($E_F \approx 0.5$ eV), is unlikely to be adequate for $\text{YBa}_2\text{Cu}_3\text{O}_7$.

In the theory of Bardeen, Cooper and Schrieffer (BCS) an effective attractive interaction, mediated by the exchange of excitations obeying Bose statistics, leads to electron pairing and superconductivity. The spectrum of these pair-mediating excitations, $\alpha^2 F(\omega)$ (where $F(\omega)$ is the density of states of the relevant excitation spectrum and α is the matrix element for coupling between the quasiparticles and the excitation spectrum) has been observed in both tunnelling⁴ and infrared^{5,6} measurements. In tunnelling, modifications of the density of states observed at energies near $\Delta + \omega_0$, where Δ is the single-particle energy gap, demonstrate coupling to excitations at a characteristic energy ω_0 . Infrared measurements, in which modulations of the conductivity occur near $2\Delta + \omega_0$, where 2Δ is the pair excitation threshold, provide a similar probe of the spectrum of pair mediating excitations⁵. The ability to probe $\alpha^2 F(\omega)$ spectroscopically has a central role in establishing phonons as the excitations that mediate pairing in conventional superconductors^{3,4}. Here we first identify the energy gap, and then examine the frequency dependence of the conductivity above the energy gap of $\text{YBa}_2\text{Cu}_3\text{O}_7$. Our measurements are

performed on $\text{YBa}_2\text{Cu}_3\text{O}_7$ (ref. 7) crystals in which the superconducting transition as measured by a.c. susceptibility is quite sharp ($\delta T_c \approx 0.2$ K, $T_c \approx 93$ K), indicating that the crystals have a high degree of electronic homogeneity.

Because of its fundamental importance, there has been considerable effort directed toward understanding the nature and magnitude of the superconducting energy gap in the layered cuprates. These materials are, of course, very anisotropic, and our interest is primarily in the response parallel to the highly conducting planes. Regarding the symmetry of the gap, the absence of temperature dependence in both the electromagnetic penetration depth^{8,9} and the infrared conductivity below ~ 50 K¹⁰ have been interpreted in terms of *s*-wave pairing. The magnitude of the energy gap has been more controversial.

Evidence for an energy gap of $2\Delta \approx 500 \text{ cm}^{-1}$ ($\approx 8 kT_c$) was originally reported for fully oxygenated $\text{YBa}_2\text{Cu}_3\text{O}_7$ ($T_c \approx 92$ K) based on measured reflectivity ratios¹¹, which exhibit a peak at 2Δ in analogy with traditional infrared gap measurements (see for example, ref. 12). Subsequent measurements of absolute reflectivity and conductivity^{13,14} also support the inference, from the infrared measurements on fully oxygenated $\text{YBa}_2\text{Cu}_3\text{O}_7$, of an energy gap of $2\Delta \approx 8 kT_c$. In lower- T_c materials of oxygen deficient $\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$ ($T_c \leq 70$ K), Thomas *et al.*¹⁵ have reported an energy gap of $2\Delta \approx 3.5 kT_c$. They have stated¹⁶, however, that the evidence for this $3.5-kT_c$ gap is near their detection limit, and that it is not seen in fully oxygenated samples. They, now, also report¹⁶ the observation of the 500-cm^{-1} feature discussed above, which they associate with a superconducting pair-breaking excitation in $\text{YBa}_2\text{Cu}_3\text{O}_7$, in reasonable agreement with our earlier work^{11,13}. An energy gap of $2\Delta \approx 8 kT_c$ has also been inferred from photoemission measurements of $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_{8-y}$ (refs 17–19), and even more recently from electron energy loss²⁰ in agreement with the original infrared identification¹¹. Interpretations of tunnelling data, that are now consistent with a large in-plane gap have also been recently proposed²¹.

In Fig. 1 the ratio of the real part of the conductivity in the superconducting state, $\sigma_s(\omega)$, at 45 K to that in the normal state, $\sigma_n(\omega)$, at 105 K is shown as a function of frequency. These conductivities are obtained from a Kramers–Kronig transformation of infrared and optical reflectivity data¹³. At high frequencies this ratio is very close to one, indicating that the transition to the superconducting state does not appreciably affect the high-frequency reflectivity or conductivity. Below $\sim 900 \text{ cm}^{-1}$ σ_s/σ_n decreases rapidly, which we interpret as the signature of the superconducting energy gap. The area that is missing (that is, where $\sigma_s(\omega) < \sigma_n(\omega)$) appears as a δ -function at $\omega = 0$ and is directly related to the electromagnetic penetration depth⁸. Ideally, in a simple isotropic superconductor σ_s/σ_n should be zero below the gap frequency, $2\Delta \approx 500 \text{ cm}^{-1}$. Because of the complex structure of $\text{YBa}_2\text{Cu}_3\text{O}_7$, which includes chains along the *b*-axis, we suggest⁵ that the finite value of σ_s/σ_n below 500 cm^{-1} may be due to contributions to $\sigma(\omega)$ that are not

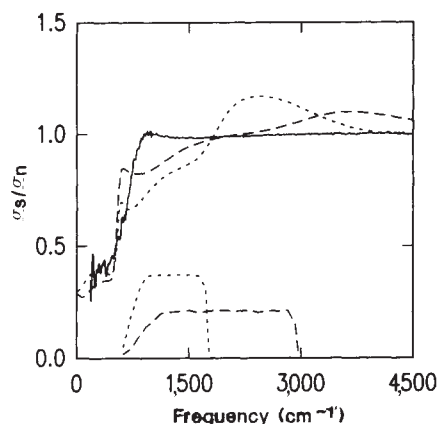


FIG. 1 The ratio of the conductivity in the superconducting state to that in the normal state for fully oxygenated ($T_c \approx 93$ K) $\text{YBa}_2\text{Cu}_3\text{O}_7$ (solid line). The smooth rise and the absence of structure above the energy gap (at $\approx 500 \text{ cm}^{-1}$) indicates a lack of evidence for a strong pairing interaction. The dotted and dashed curves show generic conductivity ratios calculated from the Eliashberg equations using the respective $\alpha^2 F(\omega - 2\Delta)$ spectra shown at the bottom. In these calculations the carrier density is $3 \times 10^{21} \text{ cm}^{-3}$ and the renormalized elastic-scattering rate is $\sim 130 \text{ cm}^{-1}$.

intrinsic to the CuO_2 planes, and therefore not of fundamental interest.

Above 500 cm^{-1} , the measured σ_s/σ_n rises rapidly and smoothly to unity. (The measured σ_s/σ_n rises at a rate consistent with weak coupling ($\lambda \leq 0.5$) and $l/\xi_0 \sim 1$, where l is the mean free path, and ξ_0 is the coherence length. This is much faster than the Mattis–Bardeen rate, which is valid for $l/\xi_0 \ll 1$.) There are a few small oscillations and a very weak maximum near $\sim 1,000\text{ cm}^{-1}$, but no evidence for the large-scale structure associated with strong coupling. Calculated spectra that demonstrate the generic behaviour associated with strong coupling are shown in Fig. 1. (If we remove the dynamic coupling, and assume a frequency-independent gap, we obtain a calculated spectrum closely resembling the data.) A modest non-superconducting fraction, which may be chain related as mentioned above, is included to mimic the behaviour below the gap. The form of the calculated spectra above 2Δ can be understood in terms of the following physics. In the superconducting state, the threshold for inelastic scattering from a mode at ω_0 is shifted by 2Δ , and enhanced because of the sharply peaked density of states just above 2Δ . This leads to a depression in σ_s/σ_n for $\omega \lesssim \omega_0 + 2\Delta$ followed by an enhancement of σ_s/σ_n at higher frequency.

Using a formulation of the Eliashberg equations for variable mean free path²², we have performed calculations using a wide variety of forms for $\alpha^2F(\omega)$. For the calculations shown in Fig. 1 we used coupling spectra, $\alpha^2F(\omega)$, that are roughly constant up to a cutoff frequency, as shown at the bottom of the figure (shifted by 2Δ). For each cutoff, the coupling strength is determined by the absolute magnitude of the measured low-temperature gap, $2\Delta \approx 500\text{ cm}^{-1}$. (The unconventional size of the measured ratio, $2\Delta/kT_c \approx 8$, presumably indicates that the transition to the normal state is not of the usual mean-field type (gap collapse due to thermal quasiparticle pair-breaking effects), hence we do not attempt to fit T_c as well.) The absence of structure in the experimental σ_s/σ_n above 2Δ suggests that no strong (retarded) pairing interaction exists in $\text{YBa}_2\text{Cu}_3\text{O}_7$. Although we have used the Eliashberg equations to calculate the curves shown in Fig. 1, our conclusions regarding the absence of a strong coupling interaction should not be construed as applicable only to the electron–phonon interaction, because the manner in which coupling influences the self-energy, and hence σ_s/σ_n , should be rather general.

To place these results in context, we discuss the behaviour of conventional superconductors. A weak-coupling superconductor such as aluminium has a $2\Delta/kT_c$ ratio near the BCS value of 3.52, and is not expected to exhibit readily observable structure in σ_s/σ_n above 2Δ . Lead, however, a strong-coupling superconductor, has $2\Delta/kT_c \approx 4.2$, and shows structure in the infrared data at $\omega \approx 2\Delta + \omega_\alpha$, where ω_α are the frequencies of the longitudinal and transverse peaks in the phonon density of states. For lead the ratio of ω_α/Δ is of the order of 3–6, whereas it is of the order of 100 for aluminium. Roughly speaking, the magnitude of the coupling-related structure in σ_s/σ_n is proportional to $(\omega_\alpha/\Delta)^{-2}$. In strongly coupled superconductors, Wada² pointed out that $2\Delta/kT_c$ would exceed the usual BCS value. They noted that, at T_c , thermally excited phonons would act as pair breakers and therefore reduce T_c , but for $T \ll T_c$, where 2Δ is measured, the density of thermal phonons with energy comparable to 2Δ is negligible so that 2Δ would not be correspondingly suppressed. Thus for ordinary electron–phonon superconductors an enhanced $2\Delta/kT_c$, and the presence of structure in the low-temperature infrared data above 2Δ , are both related to strong coupling to relatively low-lying phonons.

Apparently the conventional model does not apply to the layered cuprates. Our measurements on $\text{YBa}_2\text{Cu}_3\text{O}_7$ show a very large value of $2\Delta/kT_c \approx 8$, yet there is no evidence for any strong-coupling-related structure above the gap. Clearly, the large value of $2\Delta/kT_c$, which is unprecedented in superconductivity, cannot be explained by conventional strong coupling. The

dashed curve in Fig. 1 shows the Eliashberg result for σ_s/σ_n , using a coupling spectrum with an upper cutoff of $\omega_c \approx 2,400\text{ cm}^{-1}$ (0.3 eV). Even for this rather high cutoff ($\omega_c/\Delta \approx 10$), the effects of the coupling are quite apparent in the calculation, in contrast to the data. The amplitude of the coupling-related effects in σ_s/σ_n tends to diminish with increasing ω_c , so to generate a calculated spectrum that is not inconsistent with the infrared data one must increase ω_c still further. The inclusion of unmodulated conductivity terms, associated with either inter-band or chain-related contributions to $\sigma(\omega)$, tends to reduce the deviations of the calculated ratios from unity (because it adds terms the ratios of which are unity in σ_s/σ_n), but will not significantly alter our conclusions.

Regarding the unusually large magnitude of $2\Delta/kT_c$, one possibility is that some sort of pair-breaking scattering is suppressing T_c . To reconcile this with our present results, however, we must find some way in which pair-breaking reduces T_c , but does not modify the conductivity of the low-temperature superconducting state above 2Δ . One possibility is that coupling to low-frequency spin fluctuations could provide strong pair-breaking scattering near T_c , but that as the spin fluctuation spectrum develops a gap below T_c the pair-breaking is quenched and so does not appear prominently in σ_s/σ_n . This also provides a possible way in which to reconcile the presence of a strong dynamical interaction in the normal state, as indicated by infrared measurements²³ and t - J model calculations²⁴, with the apparent absence of strong coupling effects in the conductivity of the superconducting state.

In the original report¹¹ of the highly unusual $2\Delta \approx 8 kT_c$ energy gap it was argued, based exclusively on the magnitude of 2Δ relative to kT_c , that “Within the Eliashberg–BCS framework, this unusually large gap value suggests that $\text{YBa}_2\text{Cu}_3\text{O}_7$ is a very strongly coupled superconductor.” The present more complete examination of the Eliashberg framework indicates that such an interpretation is inconsistent with the behaviour of σ_s/σ_n above 2Δ , in which there is no evidence for strong coupling of any origin. Speculations abound as to the origin of the large ratio of 2Δ to kT_c , including fluctuation effects associated with the short in-plane coherence length or the low dimensionality, effects of pair-breaking scattering near T_c (discussed above), or the as-yet-unknown consequences of fundamentally new theories of the normal state. The implications, for both the normal state and the superconducting mechanism, of the absence of any observation of coupling-related structure in σ_s/σ_n , are not yet fully understood. It seems, however, that the layered cuprates may be fundamentally different from previously studied superconductors. □

Received 1 December; accepted 27 December 1989.

- Bednorz, J. G. & Müller, K. A. *Z. Phys.* **B64**, 189–193 (1986).
- Wada, Y. *Rev. Mod. Phys.* **36**, 253–257 (1964).
- Scalapino, D. J. in *Superconductivity* (ed. Parks, R. D.) 449–560 (Dekker, New York, 1969).
- McMillan, W. L. & Rowell, J. M. in *Superconductivity* (ed. Parks, R. D.) 561–613 (Dekker, New York, 1969).
- Joyce, R. R. & Richards, P. L. *Phys. Rev. Lett.* **24**, 1007–1011 (1970).
- Brandli, G. & Sievers, A. *J. Phys. Rev.* **B5**, 3350–3557 (1972).
- Wu, M. K. *et al. Phys. Rev. Lett.* **58**, 908–910 (1987).
- Harshman, D. R. *et al. Phys. Rev.* **B36**, 2386–2389 (1987).
- Krusin-Elbaum, L., Greene, R. L., Holtzberg, F., Malozemoff, A. P. & Yeshurun, Y. *Phys. Rev. Lett.* **62**, 217–220 (1989).
- Schlesinger, Z. *et al. Phys. Rev.* (submitted).
- Schlesinger, Z., Collins, R. T., Kaiser, D. L. & Holtzberg, F. *Phys. Rev. Lett.* **59**, 1958–1962 (1987).
- Richards, P. L. & Tinkham, M. *Phys. Rev.* **119**, 575–590 (1960).
- Collins, R. T., Schlesinger, Z., Holtzberg, F. & Feild, C. *Phys. Rev. Lett.* **63**, 422–425 (1989).
- Schutzmann, J. *et al. Europhys. Lett.* **8**, 679–684 (1989).
- Thomas, G. A. *et al. Phys. Rev. Lett.* **61**, 1313–1316 (1988).
- Orenstein, J. *et al. Preprint*, AT&T Bell Laboratories (1989).
- Manzke, R., Buslaps, T., Classen, R. & Fink, J. *Europhys. Lett.* **9**, 477–482 (1989).
- Imer, J.-M. *et al. Phys. Rev. Lett.* **62**, 336–339 (1989).
- Olson, C. G. *et al. Science* **245**, 731–733 (1989).
- Demuth, J. *et al. Preprint*, IBM T. J. Watson Research Center (1989).
- Kirtley, J. R. *Preprint*, IBM T. J. Watson Research Center (1989).
- Bickers, N. E. *et al. Phys. Rev.* (submitted).
- Collins, R. T., Schlesinger, Z., Holtzberg, F. & Feild, C. *Phys. Rev.* **B39**, 6571–6574 (1989).
- Kane, C. L., Lee, P. A. & Read, N., *Phys. Rev.* **B39**, 6880–6897 (1989).

ACKNOWLEDGEMENTS. We thank P. W. Anderson, P. M. Horn and D. M. Newns for discussions. D.J.S. and N.E.B. acknowledge the support of the NSF. D.J.S. also thanks the IBM Research Division.

Nanometre-sized diamonds are more stable than graphite

P. Badziag^{*‡}, W. S. Verwoerd^{*}, W. P. Ellis[†]
& N. R. Greiner[†]

^{*} Department of Physics, University of South Africa, Pretoria 0001, RSA
[†] Chemical and Laser Sciences Division, Los Alamos National Laboratory,
Los Alamos, New Mexico 87545, USA

DIAMONDS just 3–5 nm in diameter have recently been recovered from carbonaceous residues of detonations¹. They have also been found in meteorites², nucleate homogeneously in the gas phase³, and diamond-like films can be grown in low-pressure hydrogen⁴. Conditions are very different in these four cases, but the nearly equal sizes in both meteorites and detonations, and the necessity of nucleation centres for growth of synthetic diamonds, indicate the existence of a common underlying factor. Ultra-small diamonds, too small to be detected readily, may in fact be far more prevalent than presently realized. We arrive at this conclusion by comparing calculated heats of formation of small tetrahedral (diamond) and hexagonal (graphitic) clusters. In agreement with Nuth's discussions on diamonds in interstellar media^{5,6}, we conclude that surface energies are an important aspect in the stabilization of microcrystalline diamonds. For surface bonds terminated with hydrogen atoms, we find that diamonds smaller than ~3 nm in diameter are energetically favoured over polycyclic aromatics (the precursors to graphite), without requiring the high pressures or extreme kinetic conditions usually associated with diamonds.

Our aim is to explain observations of solid carbon in detonation products^{1,7} in which diamonds of normal structure amounted to ~20 wt% (weight per cent) of the raw soot. Gases ranging in composition from water to polycyclic aromatics account for ~25 wt% (ref. 7). The remainder of the soot is composed of graphite ribbons of the turbostratic structure in which different (0001) layers are rotationally disordered¹ and have a thickness comparable to the diameter of the associated diamonds.

With 15 wt% of the original explosive recovered as soot, a few per cent of the carbon atoms in the original explosive end up as diamond. When each surface carbon atom is bonded to hydrogen to terminate unsaturated bonds, this hydrogen accounts for only ~1% of the amount in the original explosive. Hydrogen and heteroatoms have been found to reside on the surfaces (edges) of volatile polycyclic aromatics^{7,8}, which are of the hexagonal structures considered here.

Therefore the relative stabilities of small clusters with surface bonds terminated by a plausible atomic or radical species are of interest. We began our investigation by assuming termination by hydrogen. Calculations indicate that this is favourable energetically for large single crystals of diamond⁹ and it has been established experimentally that diamond can be terminated by hydrogen^{10,11}. For hexagonal clusters with unsaturated surfaces, the heat of formation H_f is so high that in the presence of hydrogen they are very unlikely to be formed and within the present context can be ignored. We calculated H_f of several such clusters ranging from C_2 to C_{60} ; the H_f of the most stable molecule, C_{60} (soccer ball), is ~16 kcal per mol C. Even though the C_{60} molecule is particularly stable^{12,13}, its H_f is much higher than the H_f of hydrogen-saturated molecules (both hexagonal and tetrahedral).

To make our argument more quantitative, we introduce a simple model to calculate molecular energies by assigning the energies E_H to a carbon-hydrogen (C-H) bond, E_t to a tetrahedral (diamond-like) carbon-carbon (C-C) bond, and E_h to a hexagonal (graphite) C-C bond. Then the energy of a tetrahedral hydrocarbon molecule with N_C carbon atoms and N_H hydrogen

atoms may be calculated from

$$E_{\text{tot}} = -N_H E_H - \frac{1}{2} (4N_C - N_H) E_t \quad (1)$$

$$\frac{E_{\text{tot}}}{N_C} = \frac{N_H}{N_C} \left(\frac{1}{2} E_t - E_H \right) - 2 E_t, \quad (2)$$

and for the hexagonal clusters similarly,

$$\frac{E_{\text{tot}}}{N_C} = \frac{N_H}{N_C} \left(\frac{1}{2} E_h - E_H \right) - \frac{3}{2} E_h \quad (3)$$

For each molecular cluster, we calculated H_f using the MNDO (modified neglect of diatomic overlap) semi-empirical quantum chemistry program¹⁴, optimizing the C-C and C-H bond lengths but keeping the geometry restricted to hexagonal or tetrahedral symmetry. For the hexagonal series, the molecules range from benzene C_6H_6 to a ten-ring molecule $C_{32}H_{14}$. The tetrahedral molecules range from methane CH_4 and ethane C_2H_6 to adamantane $C_{10}H_{16}$ and up to a $C_{26}H_{30}$ molecule containing six adamantane cages.

In Fig. 1, we plot the binding energy, that is H_f (kcal per mol of C), as a function of the hydrogen to carbon ratio. The smallest molecules with the highest H/C ratios are shown on the right of Fig. 1, whereas the values at H/C = 0 on the left represent the infinite-crystal limit. The general trend is to form two roughly linear bands of values with the small tetrahedral molecules more stable than the small hexagonal molecules. The sp^3 molecules (tetrahedral) have a negative slope in agreement with experimental values, whereas the sp^2 clusters (hexagonal) have a slope near zero, again in agreement with measured trends. Bulk graphite is more stable than diamond and at some point the two bands must cross. In Fig. 1, the crossover occurs at H/C ≈ 0.24. To fix the lower boundaries of the bands relative to each other, we show linear regression lines fitted to our derived compact-cluster values with intercepts at 5.06 for tetrahedral and 4.11 for the hexagonal cases. According to the simple model of equations (2) and (3), these plots should be straight lines. The calculations show that the real situation is more compli-

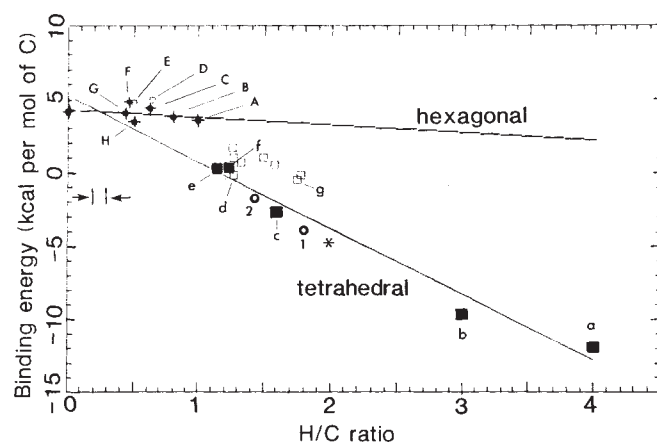


FIG. 1 Calculated binding energy in kcal per mol of carbon atoms plotted against the H/C ratio for molecules with tetrahedral and hexagonal structures. Solid squares (crosses) represent values for compact tetrahedral (hexagonal) molecules; also shown are the two solid linear-regression lines. Open squares and crosses show values for other less compact molecules. Range of experimental values are indicated by arrows. Hexagonal molecules and their total H_f in kcal mol⁻¹ are A, C_6H_6 , 21.198; B, $C_{10}H_8$, 38.1; C, $C_{16}H_{10}$, 69.826; D, $C_{22}H_{14}$, 109.87; E, $C_{28}H_{14}$, 135.26; F, $C_{30}H_{14}$, 142.73; G, $C_{32}H_{14}$, 130.1; H, $C_{24}H_{12}$, 83. Tetrahedral: a, CH_4 , -11.9; b, C_2H_6 , -19.2; c, $C_{10}H_{16}$ (adamantane), -26; d, $C_{22}H_{28}$, -2.34; e, $C_{26}H_{30}$, 7.9; f, $C_{26}H_{32}$, 10.5; g, $C_{16}H_{28}$, -7.85. Circles are points derived from ref. 15 using $H_f(H) = 52.09$ and $H_f(C_2H_2) = 54.19$ from ref. 16: 1, $C_{10}H_{18}$, -40.08; 2, $C_{14}H_{20}$ (double adamantane), -24.49. Star is for cyclohexane, C_6H_{12} , -29.43 (ref. 16).

[‡] Present address: Lawrence Berkeley Laboratory, Berkeley, California 94720, USA.

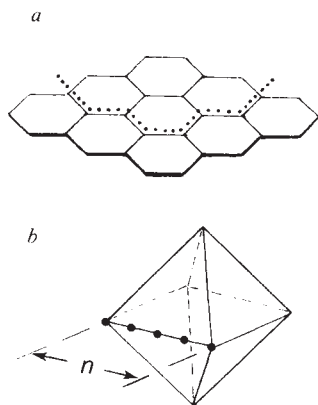


FIG. 2 *a*, Nine-ring $C_{30}H_{14}$ molecule that is cut along dotted line to give a concave five-ring $C_{22}H_{14}$ molecule for estimation of single-layer graphite bond energy. *b*, Ideal diamond octahedron with $n=5$ carbon atoms along the edge.

cated. Not all C-C bonds in any given molecule are exactly equivalent, which causes a spread of the plotted values.

To minimize this effect, we focus on the subset of those compact clusters that are most nearly spherical in the case of tetrahedral bonding or circular for the hexagonal case. Not only are these most clearly analogous to the bulk materials, but they are also those with the lowest energy for a particular H/C ratio (Fig. 1). Within this subset, our calculations are in good agreement with the values¹⁵ for two other molecules and with archival data for cyclohexane (Fig. 1).

To estimate the H_f per carbon atom of a single infinite graphite layer without the weaker interlayer interaction, we calculated H_f for a nine-ring convex molecule $C_{30}H_{14}$ and subtracted the calculated H_f of a concave five-ring molecule $C_{22}H_{14}$ (Fig. 2*a*). Because these two molecules have the same number of C-H bonds externally, the H_f difference of 32.86 kcal (4.11 kcal per carbon) may be ascribed entirely to the addition of the eight carbon atoms and their associated hexagonal bonds. The consistency of this procedure is illustrated by the close value (4.26) obtained by comparing the eight-ring $C_{28}H_{14}$ and the five-ring $C_{22}H_{14}$ molecules.

In principle, the subtraction procedure can also be used to determine the H_f per atom for an infinite diamond crystal. Within the practical limitation of about 50 carbon atoms, however, we did not find similarly related pairs of clusters with the proper structural relationship to use in estimating a reliable value for the infinite structure. Nevertheless, the intercept in Fig. 1 for infinite tetrahedral hydrocarbons is indeed higher than for the hexagonal case, although the difference of 0.95 kcal mol⁻¹ is twice the experimental difference of 0.45 kcal mol⁻¹ (ref. 16) between diamond and graphite. This discrepancy is not unreasonable in view of the spread of points on the graph, the upper limit on cluster size we were able to calculate and our simplifying assumptions.

To translate the crossover at H/C=0.24 into estimated molecular sizes, we consider a series of diamond octahedra with edges of n atoms (Fig. 2*b*). Such octahedra contain $N_C = n(4n^2 - 1)/3$ carbon atoms and $N_H = 4n^2$ hydrogen atoms to saturate surface bonds. The crystal closest to $N_H/N_C = 0.24$ has an edge of $n = 13$ with a total of 2,925 carbon and 676 hydrogen atoms for an H/C ratio of 0.23. The edge length is 3.0 nm (with a diagonal of 4.3 nm) which compares very well to the 3–5 nm microcrystals actually observed in explosion products as well as in meteorites. We estimate the size of the graphite clusters in an analogous way: symmetrical polycyclic aromatics follow the sequence C_6H_6 ($m=1$), $C_{24}H_{12}$ ($m=2$)... $C_{96}H_{24}$ ($m=4$)... $C_{6m^2}H_{6m}$, where m is the number of rings along a single edge. For these molecules the crossover at 0.24 occurs at ~ 100 carbon atoms.

Our calculations, however, cannot accurately establish the crossover to better than about a factor of two. For example, if the experimental intercept of 0.45 kcal per mol of C were chosen instead of 0.95 kcal per mol of C and if the calculated slopes in Fig. 1 were retained, the H/C crossing point would be H/C

~ 0.12 for an ~ 6.0 -nm-diameter diamond containing $\sim 21,000$ atoms and an $m=8$ polycyclic of composition $C_{384}H_{48}$. But if we plot only the cage tetrahedral molecules in Fig. 1, we obtain a crossover at H/C ~ 0.5 for an $n=6$ octahedral cluster, $C_{286}H_{144}$, 1.3 nm along the edge and aromatics $\sim C_{24}H_{12}$. Kinetics of formation and equilibration are issues with such large molecules, especially in rapid, non-equilibrium processes³ where much larger diamond is grown. Nevertheless, it remains clear that there is a crossover in stabilities.

Our results show that compact diamonds having a characteristic size of ~ 3 nm are as stable as the polycyclic precursors to graphite of the same H/C ratio regardless of pressure or kinetics. In this size range, 'metastable' diamond is not metastable at all; rather, it is the more stable polyatomic form of carbon. Kinetic conditions have to be such that either form can grow, of course, but our results imply that pressure is not necessary to form ultra-small diamonds in detonations or in cosmic environments and that extreme non-equilibrium kinetic conditions are not necessary to justify nucleation in initial stages of the vapour growth of diamond. $\square$

Received 5 September; accepted 20 November 1989.

- Greiner, N. Roy, Phillips, D. S., Johnson, J. D. & Volk, F. *Nature* **333**, 440–442 (1988).
- Lewis, R. S., Ming, T., Wacker, J. F., Anders, E. & Steel, E. *Nature* **326**, 160–162 (1987).
- Frenklach, M. et al. *J. appl. Phys.* **66**, 395–399 (1989).
- Angus, J. C. & Hyman, C. C. *Science* **241**, 913–921 (1988).
- Nuth, J. A. III *Nature* **329**, 589 (1987).
- Nuth, J. A. III *Astrophys. Space Sci.* **139**, 103–109 (1987).
- Greiner, N. R. & Hermes, R. *Proc. 9th Int. Symp. Detonation* (Naval Surface Warfare Center, Silver Spring, in the press).
- Ornellas, D. L. *Calorimetric Determination of Heat and Products of Detonation for Explosives: October 1961 to April 1982* Lawrence Livermore Natn. Lab. Rep. UCRL-52821 (1982).
- Verwoerd, W. S. *Surface Sci.* **108**, 153–168 (1981).
- Pate, B. B. *Surface Sci.* **165**, 83–142 (1986).
- Derry, T. E., Madiba, C. C. P. & Sellschop, J. P. F. *Nucl. Instrum. Meth.* **218**, 559–562 (1983).
- Kroto, H. W., Heath, J. R., O'Brien, S. C., Curl, R. F. & Smalley, R. E. *Nature* **318**, 162–163 (1985).
- O'Brien, S. C., Heath, J. R., Kroto, H. W., Curl, R. F. & Smalley, R. E. *Chem. Phys. Lett.* **132**, 99–102 (1986).
- Dewar, M. J. S. & Thiel, W. *J. Am. chem. Soc.* **99**, 4899–4907 (1977).
- Huang, D., Frenklach, M. & Maroncelli, M. *J. phys. Chem.* **92**, 6379–6381 (1988).
- Weast, R. C. (ed.) *CRC Handbook of Chemistry and Physics*, 70th edn, D50–97 (Chemical Rubber Company, Boca Raton, 1989).

ACKNOWLEDGEMENTS. Work at Los Alamos National Laboratory was supported by the U.S. Department of Energy. N.R.G. thanks the U.S. Department of Defense for support and W.P.E. thanks the Faculty of Science at the University of South Africa in Pretoria for a visiting professorship.

The origin of non-sea-salt sulphate in the Mount Logan ice core

M. C. Monaghan* & G. Holdsworth†

* Department of Geophysical Sciences, University of Chicago, Chicago, Illinois 60637, USA

† National Hydrology Research Institute, Environment Canada, Saskatoon, Saskatchewan, Canada, S7N 3H5

It has been suggested¹ that oxidized sulphur compounds might play an important part in influencing climate, as they serve as cloud condensation nuclei and thus could affect the radiative properties of clouds. Schwartz², however, finds no evidence for a climate response arising from the increased concentrations of oxidized sulphur compounds resulting from the burning of fossil fuels. He contends that such a response should be detectable if these compounds are indeed important, as they are distributed widely in the atmosphere. Measurements of sulphate concentrations^{3,4} in an ice core from Mount Logan (5,951 m), in northwestern Canada, indicate that the background concentration of non-sea-salt sulphate in snow deposited on the ice cap during the past century has remained nearly constant. Here we report ²¹⁰Pb/¹³⁷Cs ratios measured in the ice core and in soil cores collected at nearby low-altitude sites. As the ²¹⁰Pb/¹³⁷Cs ratio in

sub-micrometre aerosols decreases with altitude, and as the non-sea-salt sulphate to ^{210}Pb concentration ratios in snow deposited on Mount Logan are lower than values reported⁵ for aerosol samples collected at nearby high-latitude, low-altitude sites, our data indicate that the ice core contains sub-micrometre aerosols scavenged from the middle or upper troposphere, or both. Thus, the apparent lack of a secular increase in the non-sea-salt sulphate concentrations at Mount Logan suggests that anthropogenic oxidized sulphur compounds probably have not significantly affected a large part of the middle or upper troposphere (or both) in the remote Northern Hemisphere.

Schwartz's contention is based partly on ice-core data from the Dye-3 site on the Greenland ice cap (2,490 m; 65°12' N, 43°47' W) which indicate that the background concentration of excess sulphate in snow deposited there has tripled over the past 90 years⁶⁻⁸. (Background concentrations exclude signals of short duration and high concentration, which usually are attributed to volcanism.) In this context, it is significant that the background concentration of excess sulphate in snow deposited on the Mt Logan ice field (60°34' N, 140°30' W), at 5,340 m altitude, apparently has remained constant during the past century^{3,4}. The contrasting observations for the two sites indicate that to draw inferences from the ice-core data about the larger questions of global climate, it is necessary first to determine the relevance of the data to the general compositional structure of the atmosphere.

To do this for the Mt Logan data we have determined the specific inventories (amount per unit area) of two atmospherically derived radionuclides, ^{210}Pb (the 22.3-yr-half-life daughter of ^{222}Rn) and ^{137}Cs (the 30.1-yr-half-life fission product of nuclear-bomb detonations), both in the ice core and in soil cores collected at low-altitude sites near Mt Logan. The sample sites are shown in Fig. 1. We studied ^{210}Pb and ^{137}Cs because they are carried by sub- μm aerosols of the same size class (0.3 μm –1.0 μm median diameter) as those that carry excess sulphate⁹⁻¹¹, and because the $^{210}\text{Pb}/^{137}\text{Cs}$ activity ratio in these aerosols decreases with altitude in the atmosphere^{12,13}. Also, both radionuclides are relatively immobile in soils or ice following their deposition^{12,14-22}. Thus the ratio of their inventories ($\Sigma \text{d.p.m. cm}^{-2}$) in a soil or ice core provides a sufficient basis for calculating the time-integrated $^{210}\text{Pb}/^{137}\text{Cs}$ activity ratio in the sub- μm aerosols deposited in the core.



FIG. 1 Map showing locations of sampling sites: 1, Wolf Creek; 2, Whitehorse; 3, Kluane Lake; 4, Silver City; 5, Kluane site 2; 6, Kluane site 3; 7, White Pass; 8, Mt Logan.

We determined ^{210}Pb and ^{137}Cs inventories in the Mt Logan ice field by studying samples of ice from two adjacent ice cores, the C- and D-cores, that were extracted from the northwest col of Mt Logan at an altitude of 5,350 m in 1980 (ref. 23). In addition, we studied ion-exchange-resin-impregnated filters containing C-core trace constituents. These filters had been prepared previously for β -activity determinations²¹⁻²². The ^{210}Pb activities on the filters and in the ice were determined by isotope-dilution α -spectrometry of ^{210}Pb 's short-lived daughter, ^{210}Po (138-day half life)²⁴. Figure 2 shows the ^{210}Pb activities. To determine both the inventory of ^{210}Pb in the ice core and the efficiency with which the filters retained ^{210}Pb , we corrected these activities for decay to their values at the time of initial snow deposition using the known age stratigraphy of the ice cores²². The initial values determined from the C-core filter data, $2.29 \times 10^{-3} \text{ d.p.m. g}^{-1}$ (standard error of the mean = 5%, $n = 34$), and from the C-core ice data, $2.49 \times 10^{-3} \text{ d.p.m. g}^{-1}$ (standard error of the mean = 30%, $n = 10$), are indistinguishable within the natural variability of these activities. The ^{137}Cs activities in C-core ice deposited during the period 1950–1980 were determined from the previously reported β -activities for this period and the known density stratigraphy of the ice core. The β -emitters found in clean snow deposited during the period 1950–1980 are predominantly (>98%) ^{137}Cs and ^{90}Sr from nuclear weapon tests (presently in the proportions 1.5/1)^{12,18,19,25,26}. ^{90}Sr 's daughter ^{90}Y , ^{210}Pb and ^{210}Pb 's daughter, ^{210}Bi . The specific inventories of ^{210}Pb and ^{137}Cs in the C-core determined from these data are given in Table 1.

We determined activity profiles of ^{210}Pb , ^{226}Ra and ^{137}Cs in soil cores from six sites in the Yukon by γ -ray spectrometry of ^{137}Cs (661.65 keV), ^{210}Pb (46.54 keV) and ^{226}Ra 's short-lived daughter ^{214}Pb (351.92 keV) using a planar, intrinsic-germanium, γ -ray detector^{12,14,27}. We took soil cores only at low-altitude sites around Mt Logan because we did not find any undisturbed, nearly horizontal soil-sampling sites at high altitudes. The inventories (d.p.m. cm^{-2}) of ^{210}Pb deposited from the atmosphere was determined for each of the cores by integrating, over the entire core depth, the total activity of ^{210}Pb in excess of any ^{210}Pb activity supported by the *in situ* decay of ^{222}Rn (refs 15–17). The ^{137}Cs inventories were determined by integrating the ^{137}Cs activities in each core over its entire depth. We list excess- ^{210}Pb and ^{137}Cs inventories, their ratios ($^{210}\text{Pb}/^{137}\text{Cs}$ inventory ratios) and the altitudes of the sites in Table 1, along with the results of the study of the ice core. The listed ^{137}Cs inventories in the ice and soil cores are corrected for decay to 1 January 1980, so that they can be compared.

The $^{210}\text{Pb}/^{137}\text{Cs}$ inventory ratio in the ice core is $\geq 40\%$ lower than those in the soil cores, which indicates either that the ice and soil cores contain radionuclides scavenged from completely different regions of the atmosphere or that they contain radionuclides scavenged from a similar set of regions but in different proportions. As our principal goal is to determine the relevance of the ice-core data to the general compositional structure of the atmosphere, it is sufficient to limit the number of possible source regions to a mid- to upper-troposphere region, and to one or more regions in the lower troposphere. These are sufficient because reactive chemical species are transported and mixed over long distances in the upper and middle troposphere before their removal²⁸, whereas generally they are transported and mixed only over short distances in the lower troposphere before they are removed^{12,14,29}.

First we determine whether the characteristics of the ice-core excess sulphate and radionuclide data are similar to or different from those determined for boundary-layer aerosols collected at nearby low-altitude sites. Excess sulphate/initial- ^{210}Pb concentration ratios in the ice core, $21 \pm 13 (1 \text{ s}) \mu\text{g} (\text{d.p.m.})^{-1}$, are generally lower than those in aerosols collected at Shemya in the Aleutians, 51 to 200 $\mu\text{g} (\text{d.p.m.})^{-1}$, or at Mould Bay in the remote Canadian Arctic, 28 to 47 $\mu\text{g} (\text{d.p.m.})^{-1}$ (ref. 5). It is unlikely that orographic lifting of the local continental boundary

TABLE 1 Radionuclide data from Yukon Territory, Canada

Core	Altitude (m)	Excess ^{210}Pb inventory* (d.p.m. cm^{-2})	^{137}Cs inventory† on 1 January 1980 (d.p.m. cm^{-2})	$^{210}\text{Pb}/^{137}\text{Cs}$ inventory ratio
Wolf Creek soil core	600	5.8	7.6	0.77
Whitehorse soil core	700	7.3	7.0	1.1
Kluane Lake soil core	750	4.0	5.5	0.74
Silver Creek soil core	770	10.5	15.1	0.70
White Pass soil core	970	3.9	5.3	0.74
Kluane Site 2 soil core	1,420	5.8	7.6	0.77
Kluane Site 3 soil core	1,420	8.3	9.0	0.92
Average of soils (± 1 s)		6.5 (± 2.4)	8 (± 3.3)	0.81 (± 0.13)
Mount Logan North-west Col ice core	5,340	2.8	≥ 5.7	≤ 0.49

Soil cores up to 100 cm long were extracted with a trowel. Following collection, each soil core was subdivided into about 20 sections and dried at 110 °C. The specific activities (d.p.m. g^{-1}) of ^{137}Cs , ^{210}Pb and ^{210}Pb 's parent, ^{226}Ra (1,600-yr-half life), in the top 12 or so sections of each core were determined. For each core section, the ^{210}Pb activity supported by the decay of ^{226}Ra was determined by the product of the specific activity of ^{226}Ra , ^{220}Rn 's parent, in the core section and the average $^{210}\text{Pb}/^{226}\text{Ra}$ activity ratio in the bottom section or several sections of the core. This method presumes that any ^{222}Rn injected into soil pore space by recoil as a consequence of ^{226}Ra decay is lost to the atmosphere and that ^{210}Pb derived from the atmosphere does not penetrate to the deeper parts of the core. These presumptions are reasonable for the studied cores because: (1) the sampled soil profiles are all shallow, hence essentially all the ^{220}Rn atoms injected into soil pore space should have been able to diffuse out before their decay; (2) ^{137}Cs , which is derived exclusively from the atmosphere, is not present in the deeper parts of the cores; and (3) ^{210}Pb activities are lower than ^{226}Ra activities in the deeper sections of the cores.

* The inventory of ^{210}Pb in the ice core was calculated as the product of: (1) the combined average of initial ^{210}Pb activities determined for the ice samples, 2.33×10^{-3} d.p.m. g^{-1} (standard error of the mean = 8%, $n = 44$); (2) the average accumulation rate of ice in the cores, $37 \text{ g cm}^{-2} \text{ yr}^{-1}$ (determined previously from the profile of β -activities in the ice core²²); and (3) the mean life of ^{210}Pb , 32.2 yr.

† The specific inventory of ^{137}Cs in the core was calculated as the product of: (1) the average $^{137}\text{Cs}/(^{137}\text{Cs} + ^{90}\text{Sr} + ^{90}\text{Y})$ activity ratio in material produced during nuclear weapons tests, about 0.43 for the 1950–1980 period^{12,18,19,25,26}, and (2) the difference between the total inventory of β -emitters and twice the inventory of ^{210}Pb in the ice core (which accounts for the β -activities of both ^{210}Pb and ^{210}Bi). This calculation yields a minimum estimate for the inventory of ^{137}Cs in the ice core because we do not know the total efficiency with which the filters extracted ^{137}Cs and ^{90}Sr from the melted samples of ice, even though our measurements indicate that ^{210}Pb was extracted quantitatively, and because our measurements of the absolute activity of ^{137}Cs in leachates of several filters indicate that ^{137}Cs may have been extracted from the melted ice samples with a lower efficiency than ^{90}Sr .

layer followed by precipitation scavenging of aerosols (which might have characteristics similar to but more ^{210}Pb -rich than those sampled at Mould Bay) strongly influences the composition of snow falling on the Mt Logan ice field, because in the mountainous regions of the United States, where this occurs, $^{210}\text{Pb}/^{137}\text{Cs}$ inventory ratios in soil cores increase with altitude^{12,14}, opposite to the trend observed between the ice and soil cores on Mt Logan. (The similar $^{210}\text{Pb}/^{137}\text{Cs}$ inventory ratios in the soils sampled near Mt Logan indicate either that the aerosols deposited in the soils were scavenged from similar altitudes or that in this region the lower troposphere is well mixed.) The only site in the Northern Hemisphere Pacific region where excess sulphate/initial- ^{210}Pb concentration ratios have been determined to be as low as those in the ice core is at Midway (28° N, 177° W)⁵. Yet $^{210}\text{Pb}/^{137}\text{Cs}$ inventory ratios in soils from nearby Oahu (21° N, 158° W), yield an average $^{210}\text{Pb}/^{137}\text{Cs}$ inventory ratio of 0.93 (ref. 5), much higher than that of the ice core (0.49). Thus we conclude that it is likely that the ice core contains trace constituents scavenged not from the planetary boundary layer, but from the middle and/or upper troposphere.

We can independently define a set of altitude ranges from which the radionuclides in the ice core could have been scavenged by comparing the $^{210}\text{Pb}/^{137}\text{Cs}$ inventory ratio in the ice core with those in the soil cores. We do this by assuming that the radionuclides deposited in the soils were contained in a layer bounded below by the Earth's surface (we call this the lower region or planetary boundary layer) and that those deposited in the ice core were contained in a layer of unspecified thickness and position within a 10-km-thick troposphere (we call this the upper region). We then calculate integrated $^{210}\text{Pb}/^{137}\text{Cs}$ activity ratios in aerosols in the upper and lower regions, each over a range of layer thicknesses. Limits can then be placed on the altitude of the lower boundary of the upper region, because the ratio of the $^{210}\text{Pb}/^{137}\text{Cs}$ activity ratios in the upper and lower regions must be equal to the measured ratio of the $^{210}\text{Pb}/^{137}\text{Cs}$ inventory ratios in the ice and soil cores.

Profiles of ^{210}Pb and ^{137}Cs activities in the atmosphere have not been measured in the Arctic or sub-Arctic; ^{210}Pb and ^{90}Sr

(another product of nuclear weapons) profiles, however, have been measured at several sites in the mid-western and western United States¹³. From these we have determined, for the troposphere over the mountainous western United States, that ^{210}Pb activities (at standard temperature and pressure (STP)) drop upwards with an average scale height (the height at which the average ^{210}Pb activity (STP) is a factor of e lower than its value at the surface) of ~ 5 km, whereas time-averaged ^{90}Sr activities (STP) exhibit no trend with altitude. We use these profiles as a general indication of how ^{210}Pb and ^{137}Cs activities might vary in the atmosphere near Mt Logan so that we can calculate integrated $^{210}\text{Pb}/^{137}\text{Cs}$ activity ratios in various upper and lower regions of the troposphere.

Calculating these integrated $^{210}\text{Pb}/^{137}\text{Cs}$ activity ratios in upper and lower regions of variable thickness and comparing the ratio of these with the ratio of the $^{210}\text{Pb}/^{137}\text{Cs}$ inventory ratios in the ice and soil cores (which is ≤ 0.60) indicates, for any reasonable boundary-layer thickness (even up to 3 km), that before being deposited in the ice core, the ^{210}Pb and ^{137}Cs resided within the troposphere either entirely or predominantly above the boundary layer. Other distributions of ^{210}Pb and ^{137}Cs in the troposphere, such as those resulting from precipitation scavenging or mixing, which lead to low radionuclide activities or constant $^{210}\text{Pb}/^{137}\text{Cs}$ ratios in the boundary layer, would serve only to force higher our estimate of the altitude of the lower bound of the upper reservoir; thus the radionuclide data are consistent with our initial inference that the trace constituents in the ice core were scavenged from the middle and/or upper troposphere.

Without knowledge of the variation of excess sulphate/ ^{210}Pb concentration ratios with altitude or of the mechanism(s) that control sub- μm aerosol deposition to the ice core, we cannot completely exclude the possibility that a small quantity of sulphate-rich aerosol from the marine boundary layer could influence excess-sulphate concentrations in the ice core without modifying the core's $^{210}\text{Pb}/^{137}\text{Cs}$ inventory ratio. Nevertheless, our data are completely consistent with the radionuclides and the excess sulphate in the Mt Logan ice core having been scavenged predominantly from the middle and/or upper

troposphere. Thus, we suggest that the apparent lack of an increase in the background concentration of excess sulphate in snow deposited in the Mt Logan ice field over the past century^{3,4} indicates that during this time oxidized sulphur compounds from the burning of fossil fuels probably have not had a significant impact on a large region of the remote Northern Hemisphere middle and/or upper troposphere.

This interpretation may appear to conflict with the Dye-3, Greenland data, which indicate that a relatively remote region of the Northern Hemisphere troposphere has been considerably affected by oxidized sulphur compounds from industrial sources. However, because the $^{210}\text{Pb}/^{137}\text{Cs}$ inventory ratio in the Dye-3 core (0.96, as determined from data in ref. 19) is within the range of values determined for soils at low-altitude sites in the mid-western and northeastern United States, 1.29 ± 0.38 (1 s) (ref. 12) and because the average ratio of excess sulphate/initial- ^{210}Pb concentrations in 20-year-old and younger firn at the Dye-3 site ($43 \mu\text{g (d.p.m.)}^{-1}$) (as determined from data in refs 8, 19) is similar to but higher than in boundary-layer aerosols from Mould Bay (26 to $41 \mu\text{g (d.p.m.)}^{-1}$) and lower than in boundary-layer aerosols from the northeastern United States (216 to $306 \mu\text{g (d.p.m.)}^{-1}$)⁵, it is likely that the aerosols deposited at the Dye-3 site were scavenged from an air mass of continental-boundary-layer-like character. If this were the case, the Mt Logan and Dye-3 data would not conflict, because together they would indicate that during the past century oxidized sulphur compounds from industrial sources have been transported predominantly within the lower and/or middle troposphere. The implications for climate are, at the very least, that over the past

century oxidized sulphur compounds from the burning of fossil fuels have not directly influenced the radiative properties of clouds throughout the entire volume of the Northern Hemisphere troposphere and, most probably, that their influence has been limited predominantly to the lower and/or middle troposphere. □

Received 22 May; accepted 7 November 1989.

1. Charlson, R., Lovelock, J. E., Andreae, M. O. & Warren, S. G. *Nature* **326**, 655–661 (1987).
2. Schwartz, S. E. *Nature* **336**, 441–445 (1988).
3. Holdsworth, G. & Peake, E. *Ann. Glaciol.* **7**, 153–160 (1985).
4. Holdsworth, G., Krouse, H. R. & Peake, E. *Ann. Glaciol.* **10**, 57–62 (1988).
5. Turekian, K. K., Graustein, W. C. & Cochran, J. K. in *Chem. Oceanogr.* **10**, 51–81 (1989).
6. Herron, M. M. *J. geophys. Res.* **85**, 3052–3060 (1982).
7. Neftel, A., Beer, J., Oeschge, R. H., Zurcher, F. & Finkel, R. C. *Nature* **314**, 611–613 (1985).
8. Mayewski, P. et al. *Science* **232**, 975–977 (1986).
9. Lockhart, L. B. Jr, Patterson, R. L. & Saunders, A. W. Jr *J. geophys. Res.* **24**, 6033–6041 (1965).
10. Graustein, W. C. & Turekian, K. K. in *Precipitation Scavenging, Dry Deposition, and Resuspension* (eds Pruppacher, H. R., Semonin, R. G. & Slinn, W. G. N.) 1315–1324 (Elsevier, New York, 1983).
11. Bondietti, E. A., Papastefanou, C. & Rangarajan, C. *Am. chem. Soc. Symp. Ser. Vol. 331* (ed. Hopke, P. K.) 377–397 (American Chemical Society, New York, 1988).
12. Graustein, W. C. & Turekian, K. K. *J. geophys. Res.* **91**, 7065–7075 (1986).
13. Moore, H. E., Poet, S. E. & Martell, E. A. *J. geophys. Res.* **78**, 7065–7075 (1973).
14. Monaghan, M. C. *J. geophys. Res.* **94**, 6449–6456 (1989).
15. Benninger, L. K., Lewis, D. M. & Turekian, K. K. *Am. chem. Soc. Symp. Ser. Vol. 187*, 201–210 (1975).
16. Moore, H. E. & Poet, S. E. *J. geophys. Res.* **81**, 1056–1048 (1976).
17. Nozaki, Y., DeMaster, D. J., Lewis, D. M. & Turekian, K. K. *J. geophys. Res.* **83**, 4047–4051 (1978).
18. Volchok, H. L. in *Polluted Rain* (eds Toribara, T. Y., Miller, M. W. & Morrow, P. E.) 435–448 (Plenum, New York, 1980).
19. Koide, M., Michel, R., Goldberg, E. D., Herron, M. M. & Langway, C. C. Jr *Nature* **296**, 544–547 (1982).
20. Gaggeler, H., von Gunten, H. R., Rossler, E., Oeschger, H. & Schotterer, U. *J. Glaciol.* **29**, 165–177 (1983).
21. Delmas, R. & Pourchet, M. *Int. Assoc. hydrol. Sci. Publ.* **118**, 159–163 (1977).
22. Holdsworth, G., Pourchet, M., Prantl, F. A. & Meyerhof, D. P. *Atmos. Envir.* **18**, 461–466 (1984).
23. Holdsworth, G. in *Ice Drilling Technology USA-CRREL spec. Rep. 84-34* (eds Holdsworth, G., Kuivinen, K. C. & Rand, J.) 21–31 (1984).
24. Kharkar, D. P., Thomson, J., Turekian, K. K. & Forster, W. O. *Limnol. Oceanogr.* **21**, 294–299 (1976).
25. Cambray, R., Fisher, E. M. R., Spicer, G. S., Wallace, C. G. & Webber, T. J. *Brit. Rep. AERE-R-4687* (Atomic Energy Research Establishment, Harwell, UK, 1984).
26. Feely, H. W., Toonkel, L. & Larsen, R. *Environ. Q. Rep. EML-395*, appendix (US dept. of Energy, New York, 1981).
27. Cutshall, I. L., Larsen, R. & Olsen, C. R. *Nucl. Instrum. Meth.* **206**, 309–312 (1983).
28. Andreae, M. O. et al. *J. Atmos. Chem.* **6**, 149–173 (1988).
29. Moore, H. E., Poet, S. E., Martell, E. A. & Wilkening, M. H. *J. geophys. Res.* **79**, 5019–5024 (1974).

ACKNOWLEDGEMENTS. We thank the Arctic Institute of North America, University of Calgary for field support, the American Chemical Society for financial support, J. Kirchner for her technical help and W. Graustein, K. K. Turekian, D. MacAyeal and J. Klein for their comments.

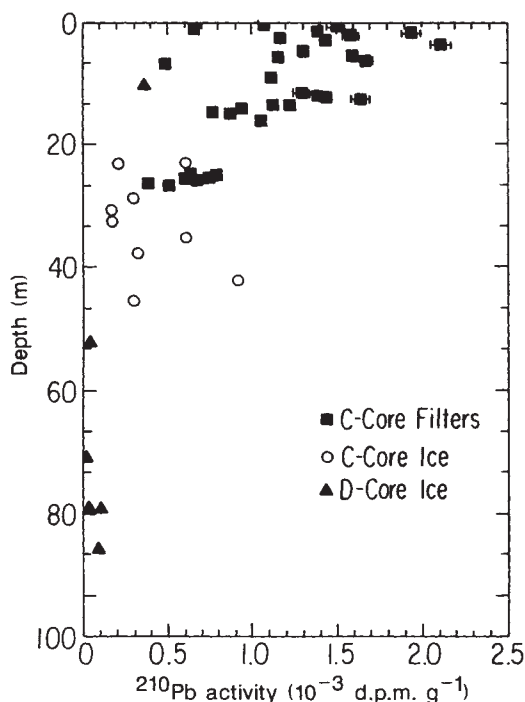


FIG. 2 ^{210}Pb activity profiles in the C-core filter samples, and C- and D-core ice from the Mt Logan ice field. The ^{210}Pb activities were determined by isotope-dilution α -spectrometry of ^{210}Pb 's daughter, ^{210}Po (138-day half life). ^{210}Po was leached from the filters with nitric acid in the presence of a known quantity of ^{208}Po (2.9-yr half life); subsequently, both isotopes were plated onto silver planchettes and their α -activities determined following the procedures, of Kharkar et al.²⁴. Before plating, the C- and D-core ice samples were melted in the presence of about 10 ml of 1N HCl containing a known quantity of ^{208}Po . The ^{210}Po activities of blanks for natural background and chemical processing were determined by measuring ^{210}Po activities on filter samples of very old (>150 yr) ice from the D-core. These blank values are negligible, about 3.5% of the values determined for recently deposited snow. The error bars indicate the analytical precision, ~3%.

Zoning of magmas by viscosity in volcanic conduits

Charles R. Carrigan* & John C. Eichelberger

Geosciences Department, Sandia National Laboratories, Albuquerque, New Mexico 87185, USA

ONE clue to the relations between different magmas may be found in the spatial relationships of the products of igneous activity. Much attention has focused on chemical zonation in the products of large ignimbrite-forming eruptions. Such deposits typically exhibit a mafic-over-silicic zonation which is interpreted as an inversion of a silicic-over-mafic layering in the source magma chamber (see, for example, ref. 1). In contrast, lava flows exhibit a silicic-over-mafic zonation, opposite to that expected. Such flows are apparently fed from conduits having a core-annular zonation, in which thin mafic margins enclose a silicic core. We argue here that this zonation develops by viscosity segregation during the simultaneous flow of two distinct magmas in the conduit. This process, rather than the pre-eruption configuration of magmas, may control the zonation pattern of eruption products when two magmas are transported simultaneously.

Walker and Skelhorn² reviewed a number of occurrences of chemical heterogeneity in igneous bodies and concluded that the typical relationship in zoned intrusions is for relatively thin

* Present address: Earth Science Department (L-206), Lawrence Livermore National Laboratory, Livermore, California 94550, USA.

mafic margins to surround a silicic core. More recently, Jones³ has done a detailed chemical analysis of a swarm of about thirty dykes exposed in the roots of the Miocene Questa caldera in northern New Mexico. All are compositionally zoned and in every case thin mafic margins enclose a thick silicic core. These authors argued that mafic magma initially opens the dyke, plating its walls, and is immediately followed by silicic magma (Fig. 1). Such an interpretation requires that the mafic component 'leap ahead' during rise of the two magmas, as the hotter and denser mafic magma probably comes from a deeper source region or from a deeper level in a stratified reservoir. Fortuitous temporal spacing of the arrival of the two magmas also seems to be required. The spacing must be long enough so that the mafic magma has begun to freeze at the wall—it will then not be abraded away by the viscous silicic intrusion. The time must be short enough that the centre of the mafic dyke retains melt, providing a zone of weakness which guides the silicic intrusion.

The alternative to sequential flow is simultaneous flow of the two components (Fig. 1). Volcanic eruptions, both explosive and non-explosive, provide clear examples of chemically different magmas flowing in the same conduit at the same time. In the highly explosive 15-km³ eruption of 1912 in the Mount Katmai region of Alaska, andesitic and dacitic magmas erupted simultaneously with rhyolitic magma to form the Valley of Ten Thousand Smokes tuff sheet⁴. Moreover, these mafic-magma-bearing pyroclastic flows may have been contemporaneous with and peripheral to a pure rhyolite ultra-Plinian column that issued from the same vent⁵. Such an arrangement would be consistent with zonation of the feeder into a rhyolitic core and mafic annular margin. At the 1,000-year-old, 1-km³ Glass Mountain

lava flow on the Medicine Lake Highland, California⁶, rhyolite overlies and is central to dacite. Two 0.1-km³ lava flows of the 600-year-old Inyo volcanic chain of eastern California consist of a crystal-rich rhyolite overlying and central to a more mafic (in terms of melt composition) and crystal-poor rhyolite⁷. A third, 0.2-km³ lava flow, discussed in detail below, consists of rhyolite overlying and central to rhyodacite. The zonation of these flows is consistent with their conduits having silicic cores and mafic margins. Magma travelling up the centre of a conduit forms the upper and central portion of a flow and magma travelling up the margin becomes the lower portion (Fig. 2).

The relationship between the zonation of a lava flow and its conduit is shown clearly in chemical profiles obtained by research drilling at Obsidian Dome in the Inyo chain (Fig. 3). Evidence indicates that the rhyolite, which comprises the upper portion of the flow and the core of the conduit, and the rhyodacite, which comprises the lower portion of the flow and the margins of the conduit, were flowing simultaneously: (1) physical continuity across chemical boundaries in both the flow and conduit; (2) fine interbanding of the two magmatic components at the boundaries; and (3) presence of both rhyolite and rhyodacite in the basal breccia of the flow, below the rhyodacite zone. The latter observation indicates that the passing flow front, from which the breccia blocks were derived, contained both magmatic components. The lava profile did not result from a pulse of rhyolite overriding rhyodacite.

Recurrence of this pattern of zonation indicates operation of some fundamental fluid mechanical process. Because the viscosity of magma is a strong function of composition, temperature and the abundance of suspended crystals, the two magmatic

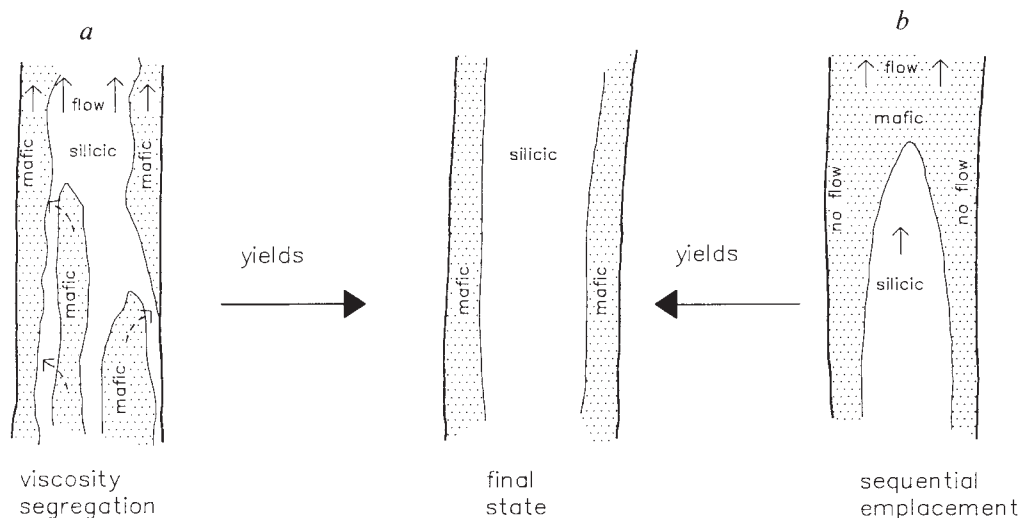


FIG. 1 *a*, Formation of core-annular flow by viscosity-segregation mechanism. Lower-viscosity mafic magma migrates to conduit margin whereas silicic magma occupies core zone. *b*, Traditional view of zoned intrusions, involving separate pulses of mafic followed by silicic magmas.

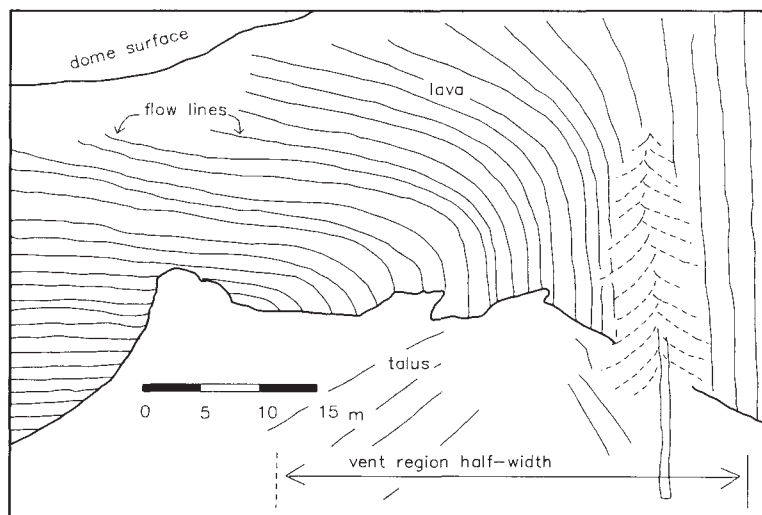


FIG. 2 Flow lines traced from photomosaic of vent region of explosively sectioned dome located near Obsidian Dome. Magma rose vertically under the centre of the dome and then flowed horizontally away from the vent.

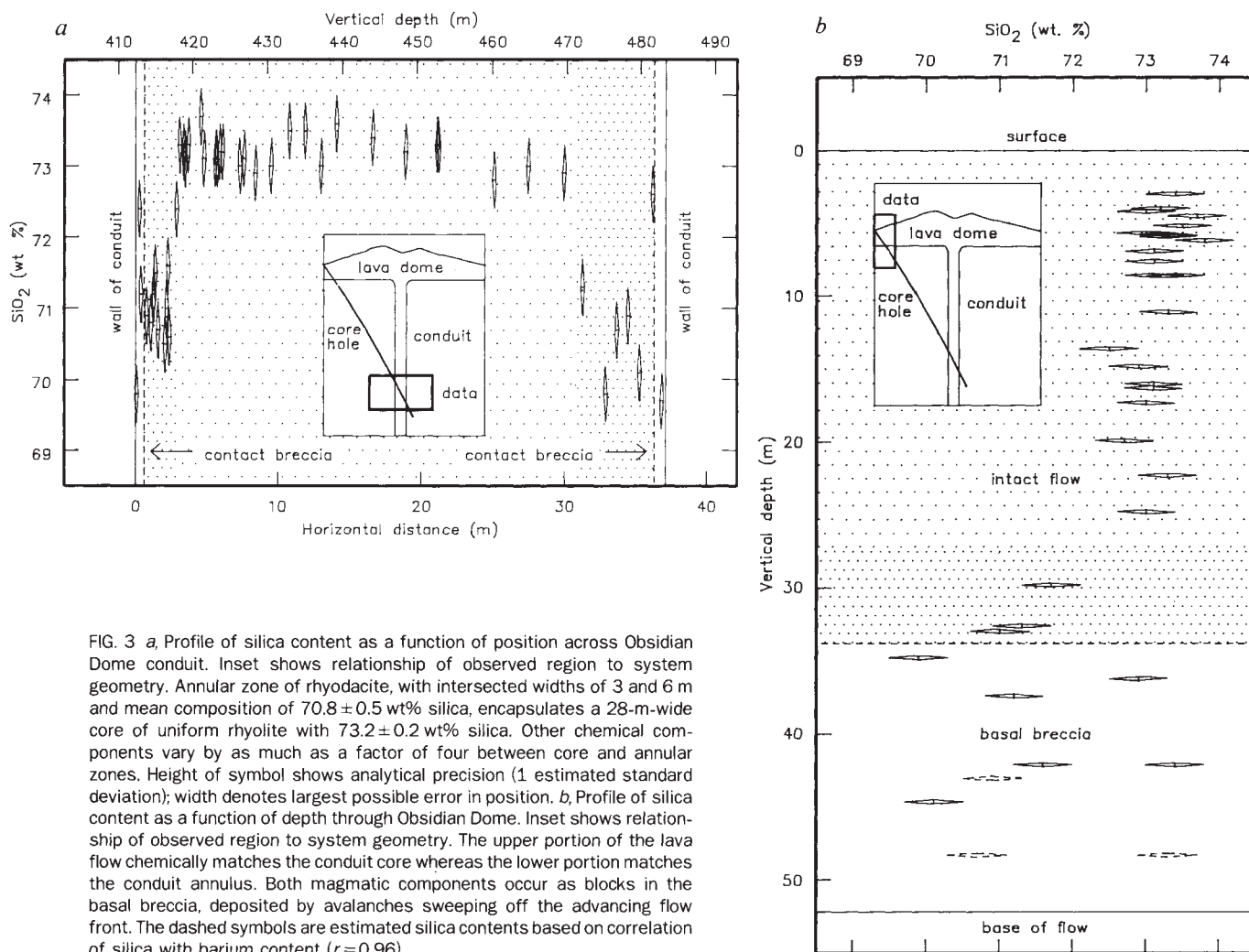


FIG. 3 *a*, Profile of silica content as a function of position across Obsidian Dome conduit. Inset shows relationship of observed region to system geometry. Annular zone of rhyodacite, with intersected widths of 3 and 6 m and mean composition of 70.8 ± 0.5 wt% silica, encapsulates a 28-m-wide core of uniform rhyolite with 73.2 ± 0.2 wt% silica. Other chemical components vary by as much as a factor of four between core and annular zones. Height of symbol shows analytical precision (1 estimated standard deviation); width denotes largest possible error in position. *b*, Profile of silica content as a function of depth through Obsidian Dome. Inset shows relationship of observed region to system geometry. The upper portion of the lava flow chemically matches the conduit core whereas the lower portion matches the conduit annulus. Both magmatic components occur as blocks in the basal breccia, deposited by avalanches sweeping off the advancing flow front. The dashed symbols are estimated silica contents based on correlation of silica with barium content ($r = 0.96$).

components that comprise the pattern would be expected to differ significantly in viscosity, with the lower-viscosity component at the margin in each case. The encapsulation of a higher-viscosity fluid by a lower-viscosity fluid in pipe flow has been noted previously in a variety of fluid mechanical studies. The goal of reducing pressure gradients in oil pipelines by the addition of water has motivated some of this work; the simultaneous transport of two polymer melts in pipes⁸ has also been studied.

Consider a liquid flowing in a vertical pipe. Continuous work is required to offset dissipation by viscosity. The region of highest shear in the flow will correspond to the highest level of dissipation. This will occur near the wall where flow velocity decreases to zero (Fig. 4a). Symmetry requires that the shear, and therefore dissipation, vanishes at the centre-line of the pipe. For a newtonian fluid, a parabolic velocity profile develops, minimizing the work required to produce a given mass flow rate. If two such fluids of differing viscosity flow in the pipe, redistribution will occur. The lower-viscosity component migrates to the wall leaving the higher-viscosity component to occupy the core (Fig. 4b). Because the rate of energy dissipation is proportional to viscosity, dissipation in the system is minimized when the lower-viscosity component flows in the region of highest shear. In effect, the annular low-viscosity fluid lubricates the rise of the viscous core. The opposite zonation, with high-viscosity fluid in the annulus, has been found to be unstable, both by linear stability theory⁹ and by laboratory experiment¹⁰.

The applicability of this model to volcanic conduits depends on the overlap of the parameter ranges appropriate to theory

and to flow of magmas. Analyses^{8,11} cover the range of Reynolds number $0 < Re \leq 10^3$, that is likely to typify conduit flows of magmas over their full composition range¹². The Reynolds number is of the order of unity for the Obsidian Dome eruption. Although most studies have dealt with immiscible fluids, the absence of surface tension between the miscible magmas should favour core-annular flow (D. D. Joseph, personal communication). The range of viscosity ratios considered in previous analyses is $1-10^2$. Calculation of magmatic viscosity from rock composition requires some inferences and assumptions. Dissolved water, which strongly affects viscosity, is inferred to have been present in the Obsidian Dome magmas at a concentration of 4 wt% (ref. 13). Suspended bubbles, which would increase viscosity, are neglected—we assume that the zoning process begins at depth before significant bubble growth occurs. Suspended crystals increase viscosity too, but the few volume per cent of crystals in Obsidian Dome magmas can be neglected¹⁴. Also because of low crystal content, bulk composition is used as melt composition. Using the model of Shaw¹⁵, we calculate the viscosity of the annular rhyodacite magma at Obsidian Dome ($T = 926 \pm 13$ °C; ref. 16) to be 1.1×10^5 P and the viscosity of the core rhyolite magma ($T = 912 \pm 11$ °C; ref. 16) to be 2.2×10^5 P. The viscosity ratio of two is appropriate for core-annular flow. For the core crystal-rich rhyolite magma ($T = 814 \pm 4$ °C; ref. 16) at the other two Inyo flows, we adjust the calculated viscosity of melt as represented by the matrix glass to include the effect of 40 vol% crystals¹⁷, and obtain a viscosity of 3.6×10^7 P. The annular crystal-poor rhyolite ($T = 920$ °C; ref. 16) is calculated to be 1.6×10^5 P. This viscosity

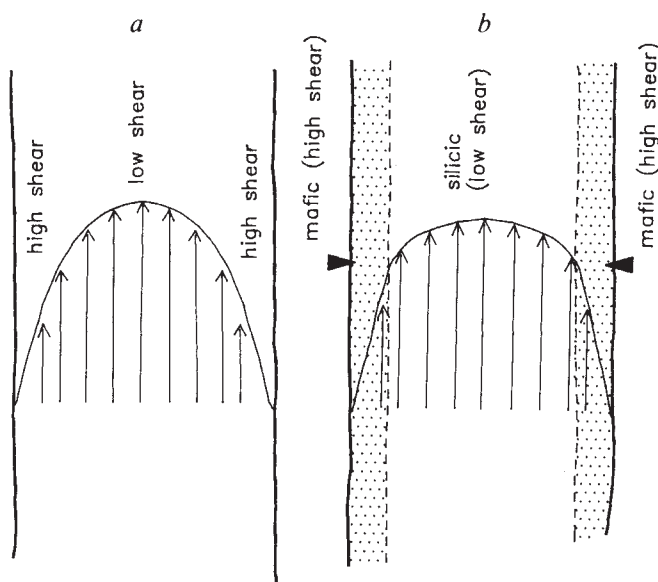


FIG. 4 *a*, Single-component flow in a conduit gives rise to a parabolic velocity profile. Regions of highest shear are near walls at which flow must vanish. Central region has lower shear. Velocity profile minimizes dissipation by viscosity for this case. *b*, For two-component flow case, dissipation is minimized by formation of lower-viscosity annular zone of high shear and higher-viscosity core of low shear. For extreme viscosity variations, shear in core almost vanishes giving rise to plug flow.

ratio of 200 implies robust core-annular segregation.

Cooling of magma at the walls of the conduit, which results in an increase in viscosity of the annular magma, tends to work against core-annular segregation. This may have little effect, however, on the dynamics of the system. At high mass fluxes early in an eruption the thermal boundary layer is thin, producing magmatic boundary temperatures¹². Later, when the flux decreases, the wall is already well heated. The cooling that does occur will produce a thin third layer at the wall having a viscosity slightly greater than the rest of the annulus. This three-layer configuration is stable provided the wall layer remains thin¹⁸. Approach toward thermal equilibrium between core and annular magmas will also decrease the viscosity contrast and may reverse it if crystallization in the annular zone results. Thermal equilibrium is unlikely to have occurred within the conduit of Obsidian Dome given the ascent time¹⁹ of a few days or less and metre-scale width of the system. Furthermore, the two magmas emerged from the conduit with distinct melt compositions, indicating that little or no crystallization resulted from their thermal contact.

The recognition of a magma-separation process that operates during transport raises a new question regarding the use of zoned eruption products to interpret relationships in the source reservoir. The zoned units discussed here could have been fed from a layered magma body, an irregularly heterogeneous body, or simultaneously from two bodies. As long as both magmatic components entered the conduit at the same time, the resulting zonation would be the same. If some of this chemical heterogeneity arises from injection of hot new magma into sluggish crustal reservoirs, as we suspect on petrological grounds^{20,21}, then the new magma acts as a lubricant as well as a trigger in facilitating the rise of high-viscosity magma to the surface. □

16. Vogel, T. A. *et al.* *J. geophys. Res.* (in the press).

17. Roscoe, R. *Brit. J. appl. Physics* **3**, 267–269 (1952).

18. Hu, H. H. & Joseph, D. D. *J. Fluid Mech.* **205**, 359–396 (1989).

19. Eichelberger, J. C., Carrigan, C. R., Westrich, H. R. & Price, R. H. *Nature* **323**, 598–602 (1986).

20. Eichelberger, J. C. *Nature* **275**, 21–27 (1978).

21. Eichelberger, J. C. *Nature* **288**, 446–450 (1980).

ACKNOWLEDGEMENTS. We thank D. D. Joseph, C. E. Hickox, S. L. Passman and L. A. Mondy for discussions and V. S. McConnell for assistance with the figures. This work was supported at Sandia and Livermore National Laboratories by the US Department of Energy.

Ignition of global wildfires at the Cretaceous/Tertiary boundary

H. J. Melosh*, N. M. Schneider*, K. J. Zahnle† & D. Latham‡

* Lunar and Planetary Laboratory, University of Arizona, Tucson, Arizona 85721, USA

† NASA Ames Research Center, Moffett Field, California 94035, USA

‡ US Forest Service, Intermountain Fire Sciences Laboratory, PO Box 8089, Missoula, Montana 59807, USA

AN impressive amount of evidence supports the proposal of Alvarez *et al.*^{1,2} that the Cretaceous era was ended abruptly by the impact of a comet or asteroid. The recent discovery^{3,4} of an apparently global soot layer at the Cretaceous/Tertiary boundary indicates that global wildfires were somehow ignited by the impact. Here we show that the thermal radiation produced by the ballistic re-entry of ejecta condensed from the vapour plume of the impact could have increased the global radiation flux by factors of 50 to 150 times the solar input for periods ranging from one to several hours. This great increase in thermal radiation may have been responsible for the ignition of global wildfires, as well as having deleterious effects on unprotected animal life.

When Alvarez *et al.*² made their impact proposal, the conventional view of impact mechanics was that crater ejecta would be thrown into the stratosphere⁵ where it would mix with the air (assuming it was dispersed as a sufficiently fine dust) and would thence spread worldwide. Although this view of the limited range of ejecta is correct for the bulk of the solid debris, which seldom attains a speed greater than about one-tenth that of the impacting projectile⁶, it has been argued⁷ that even a projectile of 10-km diameter may not generate enough fine debris to darken the globe. Furthermore, the sanidine spherules^{8,9} and shocked quartz crystals¹⁰ discovered at the boundary are too large (100–1,000 μm) to have been suspended in the atmosphere for more than a few hours. These observations, along with the

Received 13 November; accepted 1 December 1989

- Hildreth, W. *J. geophys. Res.* **86**, 10133–10192 (1981).
- Walker, G. P. L. & Skelhorn, R. R. *Earth Sci. Rev.* **2**, 93–109 (1966).
- Jones, D. M., thesis Univ. Arizona (Tucson) (1988).
- Hildreth, W. *J. Volcan. geotherm. Res.* **18**, 1–56 (1983).
- Hildreth, W. *Bull. Volcan.* **49**, 680–693 (1987).
- Eichelberger, J. C. *Geol. Soc. Am. Bull.* **86**, 1381–1391 (1975).
- Sampson, D. E. & Cameron, K. L. *J. geophys. Res.* **92**, 10403–10421 (1987).
- Joseph, D. D., Renardy, M. & Renardy, Y. *J. Fluid Mech.* **141**, 309–317 (1984).
- Hickox, C. E. *Phys. Fluids* **14**, 251–262 (1971).
- Blake, S. & Campbell, I. H. *Contr. Miner. Petrol.* **94**, 72–81 (1986).
- Preziosi, L., Chen, K. & Joseph, D. D. *J. Fluid Mech.* **201**, 323–356 (1989).
- Carrigan, C. & Schubert, G. *Eos* **69**, 1405 (1988).
- Westrich, H. R., Stockman, H. W. & Eichelberger, J. C. *J. geophys. Res.* **93**, 6503–6511 (1988).
- Milliken, W. J., Gottlieb, M., Graham, A. L., Mondy, L. A. & Powell, R. L. *J. Fluid Mech.* **202**, 217–232 (1989).
- Shaw, H. R. *Am. J. Sci.* **272**, 870–893 (1972).

worldwide occurrence of the iridium enrichment in the boundary layer, indicate a more efficient mode of global dispersal than by windblown dust.

Recent advances in the understanding of the mechanics of a large impact event^{6,11,12} show that in high-velocity impacts the expansion of the plume of vaporized projectile and target material has a larger role than previously anticipated. Theoretical studies show that the plume expands at such high speed that some of the material exceeds the Earth's escape velocity. The vapour plume comprises nearly the whole mass of the projectile along with an equal or greater contribution from the target rocks. In large impacts this plume blows aside the adjacent air and vents most of its material above the atmosphere¹³. At asteroidal impact velocities although some of this material may escape the Earth, most of it falls back into the atmosphere after following ballistic trajectories between the impact site and more distant regions of the Earth (at cometary impact velocities, however, much of this material may leave the Earth altogether¹²). In our view the worldwide distribution of iridium (and other siderophiles) is thus a reflection of ballistic emplacement of debris from the expanding vapour plume (also suggested independently by Argyle¹⁴). Of course, this material does not remain in the vapour phase for long after the impact: the cooling of adiabatic expansion condenses most of it within a few seconds. We note that estimates^{15,16} of the condensate size from the Cretaceous/Tertiary (K/T) impactor are generally in good agreement with reported sizes of the sanidine spherules.

A detailed study of vapour-plume expansion from large impacts¹² shows that in an impact of a 10^{15} – 10^{16} kg projectile on the Earth at speeds of ~ 20 – 30 km s⁻¹ most of the vaporized projectile is redeposited ballistically on the Earth at velocities in the range of 5 – 10 km s⁻¹. Although the Earth retains a smaller fraction of the mass of faster projectiles, the observed¹ global iridium abundances, 3 – 340×10^{-8} kg Ir m⁻², imply an average global deposition of projectile material of 0.06 – 6.8 kg m⁻², assuming 5×10^{-7} kg Ir per kg impactor (ref. 2). This is a lower bound on the total ejecta deposition, as the vapour plume typically contains an equal or larger mass of vaporized target. The abundance of spherules at the Petriccio section⁹ of the K/T boundary indicate a mass deposition there of 0.7 kg m⁻² from the intact spherules alone. Although large variations in the ejecta layer thickness at the K/T boundary have been observed, a reasonable global average of 3 – 4 mm (refs 14, 17, 18) implies a mass deposition of the order of 10 kg m⁻², corresponding to a total ejected mass of $\sim 5 \times 10^{15}$ kg. Locally this layer may be over 10 mm thick, yielding a mass deposition of about three times the average. We note that if the observed ejecta were deposited not by a single impact, but by a series of smaller impacts separated by an interval greater than the ballistic travel time of the ejecta (~ 1 h), then the thermal effects described below are reduced by a fraction corresponding to the fraction of ejecta contributed by each.

A very crude initial estimate indicates that the arrival of the ejecta at any point on the Earth is accompanied by impressive amounts of energy: for 10 kg m⁻² of ejecta deposited at a velocity between 5 and 10 km s⁻¹ a total energy of 1.3 – 5×10^8 J m⁻² is deposited in the atmosphere. If most of the ejecta is in the form of ~ 500 - μ m condensate particles, this energy will be emitted as thermal radiation from altitudes in the neighborhood of 70 km (see below). A standard tabulation¹⁹ of nuclear-weapons effects indicates that thermal energies of 2 – 4×10^5 J m⁻² are sufficient to ignite dry forest materials, so thermal radiation from the re-entering ejecta should be more than sufficient to start the global wildfires inferred from the soot in the boundary layer.

This question must be examined more carefully, however, because the ejecta from a large impact arrives over a considerable period of time, rather than in a brief burst, and so the rate of energy deposition must be considered. We first determine the rate of mass and energy deposition in the atmosphere as a function of distance from the impact site. We model the expand-

ing vapour plume as a hemispherical cloud with a velocity that increases linearly from zero at its centre to 10 km s⁻¹ at its outer edge (ejecta moving faster than ~ 10 km s⁻¹ escape the Earth entirely and so are of no importance for this calculation) and density distribution such that the mass is distributed uniformly in velocity. Other plausible density distributions have little effect on the final results. Based on the thickness of the observed K/T boundary layer, we assume that the total mass ejected between 5 and 10 km s⁻¹ is 5×10^{15} kg. The vapour in the cloud condenses early in its expansion and we treat the subsequent flight of the condensates ballistically. Because the ejecta is launched with a range of initial velocities and angles, it arrives at a given distance over a period of time. We divided the initially hemispherical cloud into $1,000$ bins in radius and the Earth's surface into 6° -wide angular increments from the impact site. The range and time of flight of the ejecta that fell into each increment were determined from standard ballistic formulae²⁰. The rates of mass and energy deposition between angular distances of 54 – 60° , 102 – 108° and 162 – 168° from the impact are shown in Fig. 1. We ignored the rotation of the Earth in these calculations. Although rotation may smear out the calculated pile-up antipodal to the impact site somewhat, depending on the latitude of the impact¹⁴, the present calculations are sufficiently accurate for our purpose.

It is clear from Fig. 1b that total rates of power deposition are of the order of 50 kW m⁻² or more worldwide, reaching values of 150 kW m⁻² within $\sim 7,000$ km (60°) of the impact site. Much higher values are achieved closer to the impact site and at its antipode. These energies are much larger than the solar constant, 1.37 kW m⁻². Most of this energy is radiated from the incoming ejecta as thermal energy, at temperatures between $1,000$ and $1,500$ K.

The theory of thermal radiation from entering micro-meteoroids was originally established by Whipple²¹. Unlike larger meteoroids, particles under ~ 1 mm ($1,000$ μ m) in diameter are able to radiate the heat from atmospheric entry and may survive deceleration intact. Cosmic dust particles are decelerated in just this way²². Entering ejecta with diameters < 1 mm are much smaller than the mean free path of air molecules at the altitudes where most deceleration takes place, so the flow around them is purely molecular. Using a molecular drag coefficient C_d of 2 , a 500 - μ m-diameter particle loses 50% of its initial velocity by the time it reaches an altitude of 68 km. Maximum heating takes place at an altitude $z_{\max}$,

$$z_{\max} = H \ln \left[\frac{9}{8} \frac{C_d \rho_0 H}{\delta \rho_m \cos \theta} \right] \quad (1)$$

where ρ_0 is the density of air at Earth's surface, ρ_m is the meteoroid density, δ is the spherule radius, θ is the zenith entry angle and H is the scale-height of the atmosphere ($H \approx 7$ km, ref. 23). Using a meteoroid density of $\sim 2,500$ kg m⁻³, this equation yields a $z_{\max}$ of 73 km for a 500 - μ m particle, where it reaches a maximum temperature of

$$T_{\max} = \left[\frac{1}{9e} \frac{\delta \rho_m \cos \theta}{C_d \sigma H} \nu_\infty^3 \right]^{1/4} \quad (2)$$

Where σ is the Stefan-Boltzman constant, 5.670×10^{-8} J m⁻² K⁻⁴ s⁻¹. For a 500 - μ m particle $T_{\max}$ is $1,300$ K for an entry velocity ν_∞ of 5 km s⁻¹ and $2,180$ K for 10 km s⁻¹. We note that the maximum temperature is only weakly dependent on particle size. At all altitudes the heat gained from molecular drag is radiated as thermal radiation with high efficiency because the ejecta particles have negligible heat capacity and temperatures never rise high enough to severely ablate them except at the highest speeds. Because of their small size the ejecta particles are essentially isothermal throughout entry: heat conduction is much faster (~ 1 s for 1 -mm-diameter particles) than the changes in their surface temperature as they decelerate. The silicate particles thus only undergo moderate heating—this may cause them to melt but is unlikely to evaporate any but the

fastest particles. This observation explains, first, the survival of spherules in spite of atmospheric entry at high speed and, second, spherule textures that indicate rapid cooling from a liquid phase. Although the ejecta particles originally condense from the vapour phase in the plume expanding away from the impact site, the second heating episode upon atmospheric entry melts them and produces the observed quench textures.

An entirely different approach to the thermal balance in the upper atmosphere²⁴ leads to similar results, indicating that the above temperature estimates are robust. In that analysis the total energy of the ejecta arriving over a characteristic time interval of ~ 20 min is distributed among the solid, liquid and vapour phases of the ejecta and the atmospheric gases above 60 km. The mixture is assumed to be optically thick and the temperature is calculated from energy balance. This approach yields a mean temperature in the range 800–1,100 K, somewhat lower than the maximum temperature calculated for the individual incoming particles above, but the total energy radiated is comparable.

Although the total thermal power radiated by entering ejecta is, according to Fig. 1b, of the order of 50 kW m^{-2} , not all of this radiation reaches the Earth's surface. Roughly one-half of the total is radiated upward, to space. Of the remaining 25 kW m^{-2} some is absorbed in the atmosphere by water vapour and carbon dioxide. Nevertheless, the thermal infrared 'windows' at $2.0\text{--}2.4 \mu\text{m}$ and at $3.4\text{--}4.2 \mu\text{m}$ wavelength²⁵ will, we estimate, allow roughly one-third of the total radiation to reach the Earth's surface. The centre of the $2.0\text{--}2.4 \mu\text{m}$ window corresponds to the peak of a 1,317-K blackbody spectrum, whereas the centre of the $3.4\text{--}4.2 \mu\text{m}$ window corresponds to 763 K. The thermal energy absorbed by the atmosphere is by no means negligible: with a total energy deposition of $1.4 \times 10^{23} \text{ J}$, an atmospheric heat capacity of $5 \times 10^{21} \text{ J K}^{-1}$ implies an average temperature rise of $\sim 10 \text{ K}$ if mixed uniformly. Local temperature

increases could be much greater.

A dense cloud cover will almost certainly shield underlying forests from fire: $\sim 2.5 \times 10^7 \text{ J m}^{-2}$ is required to evaporate a cloud layer with column density of $10\text{--}20 \text{ kg m}^{-2}$, appropriate for precipitating tropical clouds²⁶. This energy is comparable to the total thermal energy reaching the cloud top and should thus prevent thermal irradiation of the ground. Light cloud cover, however, with a column density $\leq 1 \text{ kg m}^{-2}$, is readily evaporated and may not provide much protection to the forests beneath.

It thus seems plausible that ejecta from the K/T impact may have given rise to a global increase of thermal radiation reaching the ground of the order of 10 kW m^{-2} over periods ranging from one to several hours after the impact. These power levels are comparable to that obtained in a domestic oven set at 'broil'. This thermal radiation may be directly or indirectly responsible for igniting the wildfires recorded in the soot layer at sites in Denmark, Spain, New Zealand^{3,4} and New Mexico²⁷. Studies of the ignition of fires by thermal radiation²⁸ have shown that, at the relatively low irradiance generated by the K/T ejecta, ignition takes place in the regime where heating of a wood surface is limited by the transport of heat away from it by convection. In general, a surface temperature near 545°C must be attained for spontaneous ignition²⁹, requiring a thermal irradiance of 29.3 kW m^{-2} , somewhat above that produced by the ejecta. However, because of the long duration of the heat source, fires may be ignited at lower irradiance levels as flammable gases are driven out of the wood or dry forest litter. This type of ignition³⁰ begins at temperatures near 380°C under irradiances of 12.5 kW m^{-2} for both wet and dry wood under prolonged (> 20 min) heating²⁹. It seems likely that local variations in either intensity of thermal radiation (some ejecta may arrive in clumps or rays of higher-than-average density, as has been observed in the far-flung ejecta of lunar craters⁶) or liability to ignition may permit fires to begin even though the predicted thermal irradiance is near the lower limit of that required for ignition of solid wood.

We thus believe that the thermal energy radiated from re-entering ejecta from the K/T impact is likely to be the cause of the global wildfires that produced the soot found in the boundary layer. Although the average irradiance is near the lower limit for ignition, we expect that fluctuations in both irradiance and flammability of materials make widespread fires a near certainty. A heat pulse of the magnitude calculated here would certainly cause widespread mortality and desiccation of plant life, leaving large amounts of standing and downed dead fuels over large areas. These areas would burn much more vigorously than green forests³¹ if ignited later by lightning. This scenario agrees well with the suggestion³ that the carbon-soot production occurred in a relatively cool, oxygen-deficient fire. Also, ballistic emplacement provides a more plausible explanation for the apparent global dispersal of the iridium anomaly and spherules, than does atmospheric dispersal, and the scenario offers a straightforward explanation for the sizes and quenched textures of the spherules themselves. Although areas shielded by thick clouds may have escaped the immediate consequences of the thermal pulse, firestorms generated elsewhere may have eventually entered these regions and ignited them. $\square$

Received 11 September; accepted 5 December 1989.

1. Alvarez, W. *Eos* **67**, 653–655 (1986).
2. Alvarez, L. W., Alvarez, W., Asaro, F. & Michel, H. V. *Science* **208**, 1095–1108 (1980).
3. Wolbach, W., Lewis, R. S. & Anders, E. *Science* **230**, 167–170 (1985).
4. Wolbach, W. S., Gilmour, I., Anders, E., Orth, C. J. & Brooks, R. R. *Nature* **334**, 665–669 (1988).
5. Emiliani, C., Kraus, E. B. & Shoemaker, E. M. *Earth planet. Sci. Lett.* **55**, 317–334 (1981).
6. Melosh, H. J. *Impact Cratering: A Geologic Process* 1–245 (Oxford University Press, 1989).
7. O'Keefe, J. D., Ahrens, T. J. & Koschny, D. in *Global Catastrophes in Earth History* 133–134 (Lunar and Planetary Institute, Snowbird, Utah, 1988).
8. Smit, J. & Klaver, G. *Nature* **292**, 47–49 (1981).
9. Montanari, A. *et al. Geology* **11**, 668–671 (1983).
10. Bohor, B. F., Modreski, P. J. & Foord, E. E. *Science* **236**, 705–709 (1987).
11. Melosh, H. J. & Vickery, A. M. *Nature* **338**, 487–489 (1989).
12. Vickery, A. M. & Melosh, H. J. in *Global Catastrophes*, *Geol. Soc. Am. Spec. Pap.* (in the press).
13. Jones, E. M. & Kodis, J. W. in *Geological Implications of Impacts of Large Asteroids and Comets on the Earth*, *Geol. Soc. Am. Spec. Pap.* 190, 175–186 (1982).

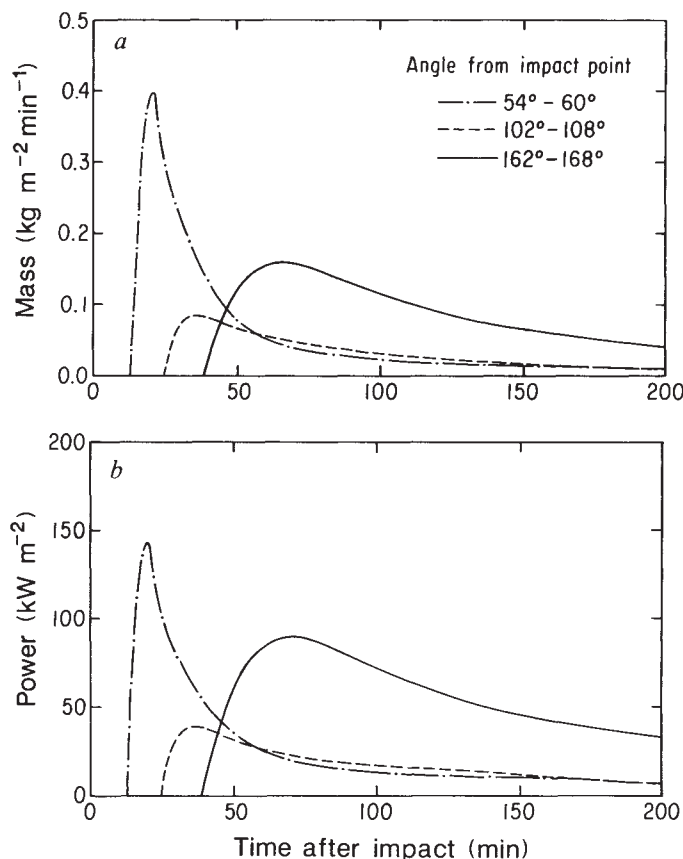


FIG. 1 a, Mass and b, power deposition in the upper atmosphere as a function of time after the ejection of $5 \times 10^{15} \text{ kg}$ at speeds between 5 and 10 km s^{-1} from a large impact on the Earth. Deposition rates are shown in three ranges of angle from the impact site.

14. Argyle, E. *Icarus* **77**, 220–222 (1989).
15. O'Keefe, J. D. & Ahrens, T. J. in *Geological Implications of Impacts of Large Asteroids and Comets on the Earth*, *Geol. Soc. Am. Spec. Pap.* 190, 103–120 (1982).
16. Melosh, H. J. in *Geological Implications of Impacts of Large Asteroids and Comets on the Earth*, *Geol. Soc. Am. Spec. Pap.* 190, 121–127 (1982).
17. Kyte, F. T., Smit, J. & Wasson, J. T. *Earth planet. Sci. Lett.* **73**, 183–195 (1985).
18. Bohor, B. F., Triplehorn, D. M., Nichols, D. J. & Millard, H. T. *J. Geology* **15**, 896–899 (1987).
19. Glasstone, S. & Dolan, P. *J. Effects of Nuclear Weapons Table 7.40* (US Departments of Defense and Energy, Washington, DC, 1977).
20. Bate, R. D., Mueller, D. D. & White, J. E. *Fundamentals of Astrodynamics* (Dover, New York, 1971).
21. Whipple, F. L. *Proc. Natn. Acad. Sci. U.S.A.* **36**, 687–695 (1950).
22. Brownlee, D. E. *A. Rev. Earth planet. Sci.* **13**, 147–173 (1985).
23. Chamberlain, J. W. & Hunten, D. M. *Theory of Planetary Atmospheres* 1–481 (Academic, Orlando, 1987).
24. Zahnle, K. J. in *Global Catastrophes*, *Geol. Soc. Am. Spec. Pap.* (in the press).
25. LaRocca, A. L. in *The Infrared Handbook* (eds Wolfe, W. L. & Zissis, G. J.) 5.1–5.132 (Office of Naval Research, Alexandria, Virginia 1978).
26. Holton, J. R. *Introduction to Dynamic Meteorology* 2nd edn, 1–391 (Academic, New York, 1979).
27. Hildebrand, A. R. & Wolbach, W. S. *Lunar planet. Sci. Conf. XX* (abstr.) 414–415 (1989).
28. Martin, S. *Proc. 10th Symp. (Int.) on Combustion* 877–896 (Williams and Wilkins, Baltimore, 1965).
29. Simms, D. L. & Law, M. *Combust. Flame* **11**, 377–388 (1967).
30. Simms, D. L. *Combust. Flame* **7**, 253–261 (1963).
31. Argyle, E. *Science* **234**, 261 (1986).

ACKNOWLEDGEMENTS. We thank A. Hildebrand for discussion of the K/T boundary-layer thickness and petrology, D. Grinspoon for discussion early in this study, R. Selkirk for information on clouds and G. Shoemaker for comments.

Evidence from carbon isotope measurements for diverse origins of sedimentary hydrocarbons

Katherine H. Freeman*, J. M. Hayes*,
Jean-Michel Trendel† & Pierre Albrecht†

* Biogeochemical Laboratories, Departments of Chemistry and of Geology, Geology Building, Indiana University, Bloomington, Indiana 47405-5101, USA

† Laboratoire de Chimie Organique des Substances Naturelles, Département de Chimie, Université Louis Pasteur, 1 rue Blaise Pascal, 67008 Strasbourg, France

THE organic matter found in sedimentary rocks must derive from many sources; not only from ancient primary producers but also from consumers and secondary producers. In all of these organisms, isotope effects can affect the abundance and distribution of ^{13}C in metabolites. Here, by using an improved form of a previously described technique¹ in which the effluent of a gas chromatograph is continuously analysed isotopically, we report evidence of the diverse origins of sedimentary organic matter. The record of ^{13}C abundances in sedimentary carbonate and total organic carbon can be interpreted in terms of variations in the global carbon cycle (see ref. 2, for example). Our results demonstrate, however, that isotope variations within sedimentary organic mixtures substantially exceed those observed between samples of total organic carbon³. Resolution of isotope variations at the molecular level offers a new and convenient means of refining views both of localized palaeoenvironments and of control mechanisms within the global carbon cycle.

We summarize the carbon isotopic compositions of individual branched and cyclic hydrocarbons extracted⁴ from the Eocene Messel Shale in Table 1. These values were obtained by integrating the mass 44, 45 and 46 ion currents from the carbon dioxide produced by continuous combustion of the chromatographic effluent (Fig. 1). The performance of this technique is limited by the chromatographic resolution, the accuracy of calibration

of the scale of isotope abundances (δ -values) and the adequacy of corrections for background carbon dioxide (largely derived from combustion of column bleed). Because well developed procedures for deconvoluting the contributions of unresolved components are not yet available, there are systematic errors in δ -values if contributions from co-eluting substances are significant. As shown in Fig. 2, such contributions lead to recognizable fluctuations in the 45/44 ion current ratio. Peaks for which there is evidence of co-eluting components are indicated in Table 1 and discussed below.

We refer the isotopic compositions to the PDB ^{13}C standard by comparison to co-injected standards; it is particularly important that these standards be well resolved. Here, the worst case is that of the standard following peak 19. Multiple analyses under varying conditions are represented by the extremes shown by Figs 1 and 2. In Fig. 2, the width of the standard peak at 10% of peak height is 21 s. Measured from baseline to baseline, the width of the corresponding oscillation in the ratio plot is 26 s. The approximately flat interval between the major oscillations in the ratio plot marks a clear separation of the isotope signals and is 25 s in width. A slightly overloaded chromatogram is shown in Fig. 1 because the large quantity of sample injected provides a clear view of minor peaks. In the corresponding ratio plot, the oscillation associated with the standard peak is 22 s in width and there is a flat interval of 10 s between the peaks. When limits of integration are selected to exclude minor components and background corrections are based on a line placed by interpolation between peak-free intervals in the chromatograms, the δ -values for peak 19 are -21.2% for data shown in Fig. 2 and -21.2% for the data shown in Fig. 1. Eight different determinations of the δ -value for peak 19 range from -19.6 to -21.5% and average -20.9% . The standard deviation of the population is 0.6% and, on that basis, the 95% confidence limits are estimated at 0.5% (s.e.m. = 0.2% , $t = 2.36$).

In a previous study of this unit, isotope analyses were performed on bulk phases (such as crude fractions of extracts) and a few compounds laboriously isolated using preparative techniques⁵. This work established carbon isotopic compositions of some Messel primary producers (eukaryotic algae, -22% ; green photosynthetic bacteria, -24%) and of lipids produced by dinoflagellates in the lake's aerobic photic zone (-25%) and by methanogenic bacteria in anaerobic portions of the water column or sediments (-30%). It was suggested that, in Lake Messel, methane rising from anaerobic zones must have been used as a carbon source by aerobic methanotrophs. Methane produced by fermentative processes in lake sediments is commonly depleted in ^{13}C (ref. 6). The appearance in Table 1 of abundant compounds strongly depleted in ^{13}C (peaks 8, 10, 11, 15, discussed below) confirms the hypothesis of methane recycling. Other features of the isotope distribution reflect the carbon pathways in, and therefore the details of, this ancient environment. Together with a recent study of functionalized lipids from the Messel Shale⁷, our results offer a perspective on Messel biogeochemistry that is different from the one provided by a recent chemical and morphological examination of Messel Shale kerogen, which indicated that the organic matter derived primarily from a single algal species⁸.

Pristane and phytane (peaks 3 and 4, Table 1) are acyclic isoprenoid biomarkers of broad geochemical interest. Their derivation has been commonly discussed in terms of oxidation or

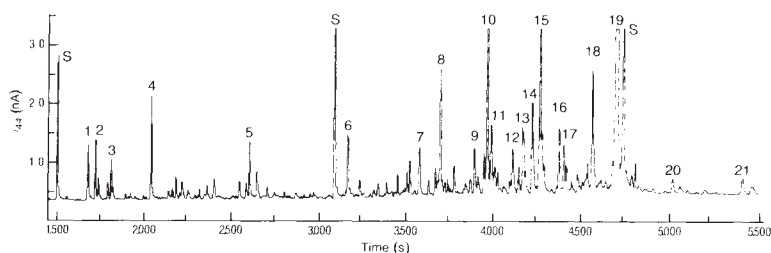


FIG. 1 Branched and cyclic alkanes extracted from the Messel Shale. Mass-44 ion current as a function of time for continuous combustion of chromatographic effluent (1-V signal corresponds to 3.3-nA ion current). Peaks are numbered as in Table 1 (S marks standard peaks). Column: 30 m $\times$ 0.32-mm inner diameter methyl silicone (Ultra-1); temperature program was 60–320 $^{\circ}\text{C}$ at 3 $^{\circ}\text{min}^{-1}$, then isothermal at 320 $^{\circ}\text{C}$ for 30 min.

TABLE 1 Carbon isotopic compositions of individual compounds

Peak	t_R^* (s)	Amount† (nmol C)	$\delta^{13}C_{\text{P}}^{\ddagger}$ (‰)	Identification
1	1,679	1.1	-22.7 ± 1.0	norpristane
2	1,722	1.0	-30.2 ± 0.3	C_{19} acyclic isoprenoid
3	1,812	0.7	-25.4 ± 1.0	pristane
4	2,040	2.0	-31.8 ± 0.8	phytane
5	2,602	1.0	-29.1 ± 0.6	C_{23} acyclic isoprenoid
6	3,161	1.3	-23.9 ± 0.6	10 β (H)-des-A-lupane
7	3,571	1.3	-24.9 ± 1.0	mixture of hydrocarbons
8	3,688	2.6	-73.4 ± 1.3	C_{32} acyclic isoprenoid
9	3,883	0.9	-24.2 ± 1.2	isoprenoid alkane
10	3,957	6.8	-49.9 ± 1.1	17 β (H)-22,29,30-trisnorhopane
11	3,977	2.0	-60.4 ± 1.8	isoprenoid alkane
12	4,100	1.6	-43.5 ± 1.0	17 α (H),21 β (H)-30-norhopane
13	4,156	2.0	~ -45	17 β (H),21 α (H)-30-norhopane§
14	4,210	2.9	~ -34	17 α (H),21 β (H)-hopane
15	4,256	6.2	-65.3 ± 1.4	17 β (H),21 β (H)-30-norhopane
16	4,364	1.8	-39.4 ± 0.8	17 α (H),21 β (H)-homohopane
17	4,392	1.3	-35.2 ± 1.4	17 β (H),21 β (H)-hopane
18	4,552	4.2	-36.6 ± 0.5	17 β (H),21 β (H)-homohopane
19	4,692	15.4	-20.9 ± 0.5	lycopane¶
20	5,010	0.5	-27.0 ± 0.4	unknown hydrocarbon
21	5,408	0.8	-28.8 ± 1.0	unknown hydrocarbon

* Chromatographic retention time (Fig. 1).

† Amounts of carbon represented by peaks in Fig. 1.

‡ $\delta = 10^3[(R_x - R_s)/R_s]$, where $R = {}^{13}C/{}^{12}C$, x designates sample, s designates PDB standard and $R_s = 0.0112372$. Entries are mean and 95% confidence interval for two to eight replicate measurements.

§ Peak contains another component, apparently an isoprenoid alkane ($\delta \sim -22\%$).

|| Peak contains another component, probably a C_{30} 4-methyl sterane. By contrast with the situation in peak 13, variations in the ratio plot indicate that δ values of the unresolved components do not differ by more than 10‰.

¶ Peak also contains minor component, apparently an acyclic isoprenoid.

reduction of phytol⁹, although the possibility of alternate origins is recognized¹⁰. Here, their isotopic compositions differ significantly and require separate origins. The δ -value for pristane fits with that expected for algal lipids (-25%). That of phytane is 5% lower, but does not differ significantly from that previously observed for ether-linked polyisoprenoids in these sediments (-29.8% , ref. 5). Together with results of stereochemical investigations¹¹, these observations strongly suggest derivation of phytane principally from lipids of methanogenic bacteria. The C_{19} acyclic isoprenoid in peak 2 and the C_{23} acyclic isoprenoid (peak 5) probably share this source. The 10 β (H)-des-A-lupane^{12,13} may derive from photochemical or bacterial photo-mimetic decomposition of pentacyclic triterpenoids from higher plants¹², and thus may record their isotopic composition. The δ -value exceeds that previously observed for leaf material from Eocene land plants (-27.5%) and for isoarborinol (-28.1%) isolated from the Messel Shale³. It is possible: (1) that both isoarborinol and the lupane are of higher-plant origin, the latter deriving from ${}^{13}C$ -enriched aquatic macrophytes¹⁴ and the former from land plants, or (2) that the lupane is representative of terrestrial plants that were, like some modern tropical plants, relatively enriched in ${}^{13}C$ (ref. 3). In the latter case, as previously suggested¹⁵, a microbial origin is likely for isoarborinol. Norpristane (peak 1), the compound in peak 9, and possibly lycopane, may all have the same source as the des-A-lupane. Other compounds possibly related to primary producers are the late-eluting peaks 20 and 21, which have isotopic compositions consistent with a terrestrial-plant origin.

Although its structure is not known in detail, the isotopic composition of the C_{32} acyclic polyisoprenoid in peak 8 indicates that it was derived from methanotrophic bacteria. A normal kinetic isotope effect of up to 25‰ is associated with the assimilation of methane by these organisms¹⁶, which will, therefore, be even more strongly depleted in ${}^{13}C$ than their carbon source. The extent of depletion depends on the efficiency with which the bacteria are able to capture the methane. In a natural, open system, that efficiency is likely to have been low, and peak 8 may be as much as 25‰ depleted in ${}^{13}C$ relative to the methane. Therefore, we now estimate $-48 \geq \delta(CH_4) \geq -73\%$ in Lake

Messel, in excellent agreement with observations of $\delta(CH_4)$ in modern lacustrine systems⁶.

Other compounds so strongly depleted in ${}^{13}C$ that they are probably derived, at least in part, from methanotrophs are the C_{29} hopane^{4,17} in peak 15, the C_{27} hopane^{4,17} in peak 10 and the acyclic polyisoprenoid in peak 11. As polyisoprenoids, these would derive from the same biosynthetic pathway as the compound in peak 8. Had they been synthesized by the same organism, they would have the same isotopic composition. The significant enrichment in ${}^{13}C$ relative to peak 8 indicates either an origin from a different methanotroph or, more likely, partial derivation from nonmethanotrophic sources. This is particularly clear in the case of 17 β (H),21 β (H)-30-norhopane (peak 15), which is the most likely degradation product (by oxidation and decarboxylation)¹⁸ of the C_{35} aminopentol that is the most abundant hopanoid in *Methylococcus capsulatus* and in *Methylomonas* sp.^{18,19}. This same C_{29} hopane is a common constituent of many sediments that do not bear similar evidence of methanotrophy^{17,20}, and can derive from a variety of bacterial sources^{20,21}.

The ubiquitous bacterial hopanes comprise the remaining entries in Table 1. Notably, these include the $\alpha\beta$ - and $\beta\alpha$ -stereoisomers²² of the ${}^{13}C$ -depleted $\beta\beta$ - C_{29} -hopane in peak 15.

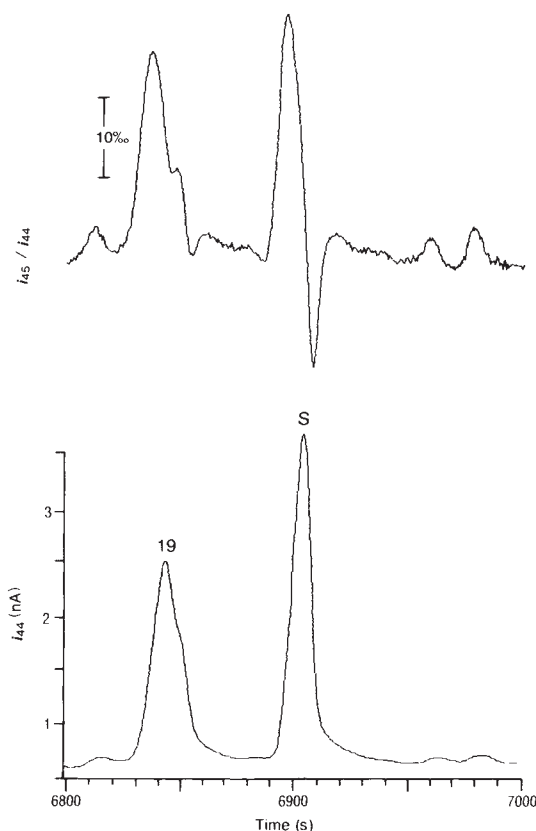


FIG. 2 The lower plot is an expanded view of the region around peak 19 in a chromatogram of the same sample shown in Fig. 1. In this case, the total sample load was fourfold lower and the rate of the temperature programme was 2° min^{-1} . Retention times were longer and peaks were wider, but resolution improved. The upper plot shows the mass-45/mass-44 ion current ratio. Because alkanes containing one or more ${}^{13}C$ atoms migrate through the chromatographic system more rapidly than those containing only ${}^{12}C$, ${}^{13}CO_2$ (mass 45) precedes ${}^{12}CO_2$ (mass 44) at the ion source. For the standard peak shown here (S), this causes the ratio signal first to increase sharply, indicating that the leading edge of the standard peak is enriched in ${}^{13}C$ relative to the background, then drop to values below the baseline as the later, ${}^{12}C$ -rich portion of the peak is eluted. Because peak 19 is relatively enriched in ${}^{13}C$, the negative portion of its ratio oscillation is small. The appearance of an extra inflection in the ratio plot for peak 19 indicates the presence of more than one component or, possibly, a mixture of diastereomers.

Isotope differences indicate that the $\alpha\beta$ - and $\beta\alpha$ -isomers cannot have derived from epimerization of average $\beta\beta$ -C₂₉-hopane. Through its mechanism or environment²³⁻²⁶, the process of epimerization must have operated selectively on a precursor pool that happened to be isotopically distinct. Isotopic compositions of the remaining hopanes range from -34 to -39%. Although even this variation may reflect minor methanotrophic inputs, it is more useful to consider these materials as end-members. What organisms might have produced them and how might their isotopic compositions be explained? One clue is furnished by the isotopic composition of nitrogen in Messel kerogen. Analysis yields $\delta^{15}\text{N} = +6.8\%$ relative to air (J. W. Collister, personal communication). This ^{15}N enrichment indicates active recycling of nitrogen, probably involving ammonia-oxidizing bacteria²⁷. Such organisms are microaerophilic chemoautotrophs known to synthesize hopanoids²¹. In Lake Messel, these bacteria would have grown at the oxycline and used dissolved carbon dioxide as their carbon source ($\delta \sim -14\%$, estimated from isotopic composition of green photosynthetic bacteria and from the isotope effect associated with carbon fixation^{28,29}). Ammonia-oxidizing bacteria use the C₃ pathway of carbon fixation. Depending on the concentration of dissolved carbon dioxide, they will be depleted in ^{13}C relative to their carbon source by up to 27% (ref. 30). Depletions of $\sim 20\%$ are plausible and would yield $\delta(\text{whole organisms}) = -34\%$, $\delta(\text{hopanoids}) \approx -37\%$. Chemoautotrophic bacteria are, thus, a possible source of biomarker lipids depleted in ^{13}C relative to total organic carbon (a similar situation will arise in marine sediments if, as is likely, sulphide-oxidizing bacteria³¹ use ^{13}C -depleted carbon dioxide at the base of the aerobic zone).

We cannot exclude heterotrophic bacteria as a source for the ^{13}C -depleted hopanes. Bacterial reworking of primary biological inputs must have been particularly important in anaerobic portions of the water column and in the sediments, in which a fermentative community produced considerable quantities of methane. Unfortunately, little is known regarding intermolecular isotopic fractionations within such communities. In an environment in which δ -values of fresh biological material ranged from -20 to -75%, almost any composition might be found, particularly if seasonal factors affected sources of organic material. Moreover, a compound with $\delta = -55\%$ might derive, not from a 1:1 mixture of -35 and -75% endmembers, but from synthesis by an organism that used both ^{13}C -rich and ^{13}C -poor carbon sources.

Although uncertainties in isotope ratios would be decreased if we used preliminary chromatographic steps to prepare simpler mixtures, the isotopic differences evident in Table 1 demonstrate the value of molecular isotope analyses for the study of natural systems. From the viewpoint of biogeochemistry, it seems that sedimentary organic compounds must increasingly be viewed as products of a microbial community, not merely as residues of primary production. □

19. Neunlist, S. & Rohmer, M. *Biochem. J.* **231**, 635-639 (1985).
20. Ourisson, G., Rohmer, M. & Albrecht, P. *Pure appl. Chem.* **51**, 709-729 (1979).
21. Ourisson, G., Rohmer, M. & Poralla, K. A. *Rev. Microbiol.* **41**, 301-333 (1987).
22. Van Dorsselaer, A., Albrecht, P. & Ourisson, G. *Bull. Soc. chim. Fr.* 165-170 (1977).
23. Quirk, J. M., Wardrop, A. M. K., Wheatley, R. E. & Maxwell, J. R. *Chem. Geol.* **42**, 25-43 (1984).
24. Michaelis, W., Richnow, H. H. & Jenisch, A. *Sci. Total Envir.* **81/82**, 41-50 (1989).
25. Seifert, W. K. & Moldovan, J. M. in *Advances in Organic Geochemistry 1979* (eds Douglas, A. G. & Maxwell, J. R.) 229-237 (Pergamon, Oxford, 1980).
26. Ries-Kautt, M. & Albrecht, P. *Chem. Geol.* **76**, 143-151 (1989).
27. Collister, J. W., & Hayes, J. M. *Bull. U.S. geol. Surv.* (in the press).
28. Sirevag, R., Buchanan, B. B., Berry, J. A. & Troughton, J. H. *Arch. Microbiol.* **112**, 35-38 (1977).
29. Fry, B. *Limnol. Oceanogr.* **31**, 79-88 (1986).
30. Popp, B. N., Takigiku, R., Hayes, J. M., Louda, J. W. & Baker, E. W. *Am. J. Sci.* **289**, 436-454 (1989).
31. Kepkay, P. E., Cooke, R. C. & Novitsky, J. A. *Science* **204**, 68-69 (1979).

ACKNOWLEDGEMENTS. We thank S. A. Studley for technical assistance, M. P. Ricci for development of isotope-ratio-monitoring software, and M. Rohmer and J. W. Collister for discussions. We also thank NASA, Chevron Oil Field Research Corporation and Finnigan MAT for support.

Magnetic iron-sulphur crystals from a magnetotactic microorganism

Marcos Farina*, Darci Motta S. Esquivel† & Henrique G. P. Lins de Barros†‡

* Instituto de Biofísica, UFRJ, Cidade Universitária, Ilha do Fundão, Rio de Janeiro 21941, Brazil

† Centro Brasileiro de Pesquisas Físicas, CBPF/CNPq, Rua Xavier Sigaud 150, 22290 Urca, Rio de Janeiro, Brazil

IN all magnetotactic microorganisms studied so far, the geomagnetic field is detected by magnetic particles with a permanent magnetic moment. These are crystallites enveloped by a membrane that forms the magnetosome, a specialized organelle common to magnetotactic cells^{1,2}. To date, the magnetic crystallite of magnetotactic bacteria has been found to be magnetite, an iron-oxygen mineral³⁻⁷. Here we report the discovery of magnetic iron-sulphur crystals in a highly motile multicellular aggregate of bacteria found in brackish water with sulphide-rich sediments. The iron sulphide crystals are enveloped by amorphous or weakly crystalline regions rich in iron and oxygen, and these regions are surrounded by a membrane forming the magnetosome. The oxygen-rich region may be involved in growth of the iron sulphide crystals. The magnetosomes are found in planar groups inside the cytoplasm of each cell in the aggregate. Magnetic iron sulphide such as we describe here, which is probably pyrrhotite, may be a source of remnant magnetization in sediments and soils.

We have studied sediments from marine lakes at latitudes between 7° and 27° in the Southern Hemisphere; as well as coccus bacteria we found a highly motile flagellar magnetotactic multicellular aggregate (MMA) of diameter between 5 and 10 μm , which orientates in the geomagnetic field. The typical magnetic moment is 10^{-11} e.m.u. These organisms are composed of about twenty double individual cells bounded by two membranes, and they contain more than 1,000 crystals that are not completely orientated. MMAs were collected at depths varying from ~ 20 cm to > 1 m in marine or brackish water at the interface between water and sediment. Their fine-structure and behavioural response have been described⁸.

Differential interference contrast optical microscopy and scanning electron microscopy (SEM) show that each aggregate is made up of cells morphologically arranged in a helicoidal-like distribution. In low-salinity medium (distilled water added to the sample), living organisms disaggregate into individual components that have neither magnetic orientation nor movement. Living individual components have never been observed. SEM and transmission electron microscopy (TEM) were used in conjunction with fixation techniques previously described⁹.

‡ To whom correspondence should be addressed.

Received 3 July; accepted 21 November 1989.

1. Matthews, D. E. & Hayes, J. M. *Analyt. Chem.* **50**, 1465-1473 (1978).
2. Knoll, A. H., Hayes, J. M., Kaufman, A. J., Swett, K. & Lambert, I. B. *Nature* **321**, 832-838 (1986).
3. Deines, P. in *Handbook of Environmental Isotope Geochemistry* Vol. 1A (eds Fritz, P. & Fontes, J. C.) 329-406 (Elsevier, Amsterdam, 1980).
4. Kimble, B. J. et al. *Geochim. cosmochim. Acta* **38**, 1165-1181 (1974).
5. Hayes, J. M., Takigiku, R., Ocampo, R., Callot, H. J. & Albrecht, P. *Nature* **329**, 48-51 (1987).
6. Whiticar, M. J., Faber, E. & Schoell, M. *Geochim. cosmochim. Acta* **50**, 693-709 (1986).
7. Robinson, N., Eglinton, G., Cranwell, P. A. & Zeng, Y. B. *Chem. Geol.* **76**, 153-173 (1989).
8. Goth, K., deLeeuw, J. W., Puttmann, W. & Tegelaar, E. W. *Nature* **336**, 759-761 (1988).
9. Didyk, B. M., Simoneit, B. R. T., Brassell, S. C. & Eglinton, G. *Nature* **272**, 216-222 (1978).
10. ten Haven, H. L., deLeeuw, J. W., Rulkötter, J. & Sinnighe Damste, J. S. *Nature* **330**, 641-643 (1987).
11. Risatti, J. B., Rowland, S. J., Yon, D. A. & Maxwell, J. R. *Org. Geochem.* **6**, 93-104 (1984).
12. Corbet, B., Albrecht, P. & Ourisson, G. *J. Am. chem. Soc.* **102**, 1171-1173 (1980).
13. Trendel, J.-M. et al. *Tetrahedron* **45**, 4457-4470 (1989).
14. Osmond, C. B., Valaane, N., Haslam, S. M., Uotila, P. & Roksandic, Z. *Oecologia* **50**, 117-124 (1981).
15. Ourisson, G., Albrecht, P. & Rohmer, M. *Trends Biochem. Sci.* **7**, 236-239 (1982).
16. Coleman, D. D., Risatti, J. B. & Schoell, M. *Geochim. cosmochim. Acta* **45**, 1033-1037 (1981).
17. Ensminger, A., Van Dorsselaer, A., Spyckerelle, Ch., Albrecht, P. & Ourisson, G. in *Advances in Organic Geochemistry 1973* (eds Tissot, B. P. & Biener, F.) 245-260 (Technip, Paris, 1973).
18. Zundel, M. & Rohmer, M. *FEMS Microbiol. Lett.* **28**, 61-64 (1985).

TABLE 1 Electron-diffraction data for iron-sulphide inclusions compared with standard pyrrhotite 4C monoclinic and magnetite

Data†	Assignment*	
	Pyrrhotite, 4C monoclinic	Magnetite
5.676	5.67	—
—	—	4.85
3.504	3.42	—
3.106	3.11	—
2.954	2.966	2.967
2.862	2.847	—
2.686	2.671	—
—	—	2.532
2.457	2.435	—
2.122	2.149	—
—	—	2.099
1.891	1.919	—
—	—	1.715
1.601	1.601	1.616
1.494	1.484	1.485

* Standard mineral d spacings in Å. X-ray powder diffraction file: pyrrhotite 4C monoclinic (17.200) magnetite (19.629).

† Experimental d spacings in Å.

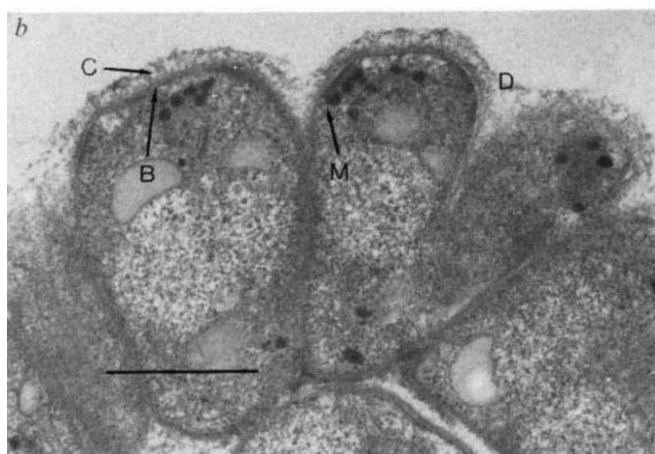
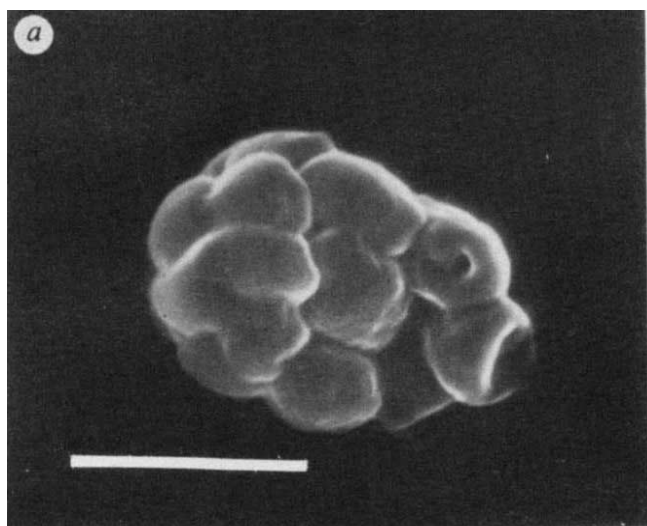


FIG. 1 *a*, Partially disrupted magnetotactic multicellular aggregate (MMA) observed by scanning electron microscopy. Note the individual cells arranged in this typical organization. MMA was fixed after 10 min in distilled water. Scale bar, 3 μ m. *b*, Thin section of MMA observed by transmission electron microscopy. Membrane specialization, A; Inner membrane, B; outer membrane, C; cellular coat, D; magnetosome, M. Scale bar, 1 μ m.

SEM of the partially disrupted organism shows cells with a complex organization which is similar in several organisms (Fig. 1*a*). Fixed samples embedded in EPON, ultra-thin sectioned and analysed by TEM, indicate that there is an interdependence between the double-membrane individual components. Electron-dense regions (EDR) with a roughly geometrical shape are observed in almost all individual components, but they are not homogeneously distributed. No cell nucleus was observed and each component seems to be a prokaryotic cell (Fig. 1*b*). These and earlier observations⁸⁻¹⁰ indicate that MMA represents a

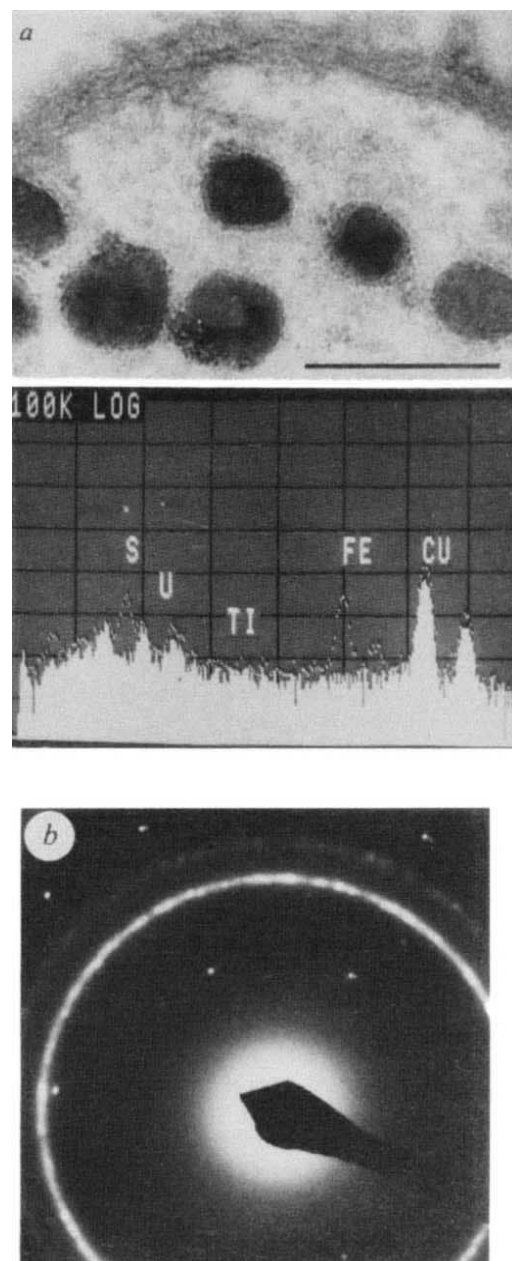


FIG. 2 *a*, Comparison by energy dispersive X-ray microanalysis of magnetosome (dotted line) and cytoplasm regions (white background). Note the low signal of iron or sulphur in the cytoplasm in contrast to the high density concentration of these elements in the magnetosome. The Cu peak comes from the grid bar. A JEOL 100 CX microscope with ASID 4D attachment and ORTEC X-ray microanalyser system was used. Top: EDR from which the spectrum was obtained, showing that crystals are surrounded by a unit membrane-like structure. Scale, 200 nm. *b*, Typical electron-diffraction pattern of some crystalline electron-dense regions superimposed on rings of vacuum-evaporated gold for calibration. A JEOL 100 CX microscope was used for selected area diffraction.

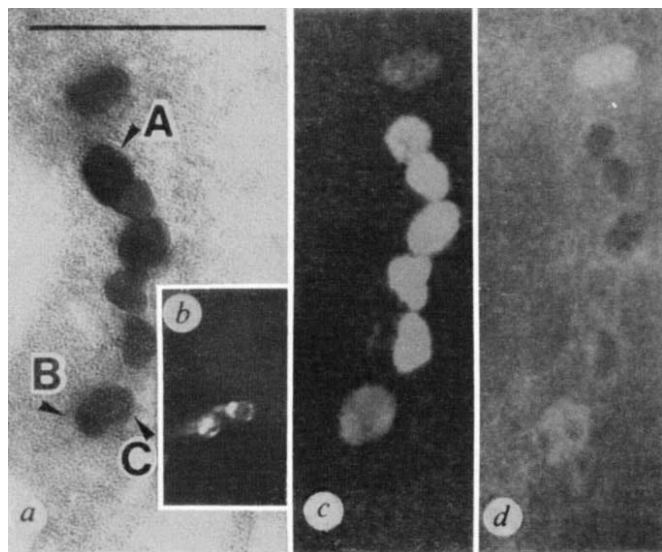


FIG. 3 *a*, Bright-field images of 7 electron-dense regions (EDR). Two crystals are observed in Bragg diffraction conditions (denoted by A and B). Crystal C is not seen in this condition. *b*, Dark-field image of the same region as in *a*. The two bright regions are crystals B and C growing inside one electron-dense region. The sample holder was appropriately tilted to obtain this image because crystal C was not in the Bragg condition in situation *a*. *c*, Iron electron spectroscopic images (ESI) from the same region as in *a*. Note that the region surrounding crystals B and C has a low iron concentration and that the EDR has differential iron concentration. The highest concentration of iron occurs exactly over the crystalline region. Maximum energy, 716 eV; minimum energy, 690 eV; slit, 20 eV. *d*, Oxygen ESI from the same sample showing that crystalline regions contain non-oxygen crystals. The highest concentrations of oxygen are observed around crystallites. Oxygen-rich regions are amorphous because no Bragg condition for diffraction was found. All the other EDR are crystalline because it was possible to find conditions for diffraction similar to that indicated by the arrow A in *a*. Maximum energy, 550 eV; minimum energy, 500 eV; slit, 20 eV. Scale bar, 1.0 μm . A CEM-902 Zeiss microscope was used.

bacteria colony. EDR were identified as magnetosomes, with typical dimensions ranging between 75 to 100 nm. By tilting the sample, some magnetosomes are seen by TEM to be flat and bounded by membranes⁸.

Energy dispersive X-ray microanalysis shows that EDR are rich in iron and sulphur¹¹, although we could not establish a stoichiometric formula (Fig. 2*a*). Whole organisms when disrupted by distilled water, placed on formvar-covered grids and observed by TEM present thin regions that make it possible to obtain electron-diffraction patterns of EDR. From an average of more than 30 electron diffraction patterns from different EDR, we deduced that these are crystals giving patterns different from magnetite (Fig. 2*b*; Table 1). By tilting the sample holder, we could obtain images from EDR under Bragg diffraction conditions, when we found crystalline regions inside the EDR. Some EDR present more than one crystalline region (Fig. 3*a, b*). Electron spectroscopic images obtained with a CEM-902 Zeiss microscope¹² show that iron is concentrated in all the EDR. Comparing these images with those from diffraction contrast, we conclude that regions of higher iron concentration are crystalline, with no oxygen in their structure (Fig. 3*c, d*). Iron-oxygen-rich regions are found in amorphous areas surrounding the growing crystals and are probably related to intermediate compounds responsible for the biomineralization process.

Electron diffraction patterns were then evaluated with respect to various magnetic minerals, particularly magnetite and pyrrhotite. The match was poor with all magnetic minerals (including magnetite), but there was some agreement with standard monoclinical pyrrhotite 4C tables¹³ and with patterns from natural magnetic pyrrhotite samples. Although we have no single

piece of evidence that unequivocally identifies pyrrhotite, the electron diffraction patterns and all our earlier observations indicate that these microorganisms biomineralize pyrrhotite. Amorphous-like regions may act as a mother matrix to generate magnetic crystallites and to protect their growth.

Biogenic pyrrhotite and biogenic magnetite can contribute to the magnetization of sediments and mineral reservoirs found in soils. The discovery of a magnetic iron-sulphide biogenic material, probably pyrrhotite as we report here, should enhance our understanding of diagenesis. □

Received 17 August; accepted 23 November 1989.

1. Blakemore, R. P. *Science* **190**, 377–379 (1975).
2. Frankel, R. B., Blakemore, R. P. & Wolfe, R. S. *Science* **203**, 1355–1356 (1979).
3. Matsuda, T., Endo, J., Osakabe, N., Tonomura, A. & Arai, T. *Nature* **302**, 411–412 (1983).
4. Towe, K. M. & Moench, T. T. *Earth planet. Sci. Lett.* **52**, 213–220 (1981).
5. Mann, S., Frankel, R. B. & Blakemore, R. P. *Nature* **310**, 405–407 (1984).
6. Bazylinski, D. A., Frankel, R. B. & Jannasch, H. W. *Nature* **334**, 518–519 (1988).
7. Blakemore, R. P. *A. Rev. Microbiol.* **36**, 217–238 (1982).
8. Farina, M., Lins de Barros, H. G. P., Esquivel, D. M. S. & Danon, J. *Biol. Cell.* **48**, 85–86 (1983).
9. Lins de Barros, H. G. P. & Esquivel, D. M. S. in *Magnetite Biomineralization and Magnetoreception in Organisms* (eds Kirschvink, J. L. et al.) 289–309 (Plenum, New York, 1985).
10. Esquivel, D. M. S., Lins de Barros, H. G. P., Farina, M., Aragão, P. H. A. & Danon, J. *Biol. Cell.* **47**, 227–234 (1983).
11. Farina, M., Sollorzano, G. & Vieira, G. J. *Proc. Xlth Int. Cong. on Electron Microscopy, Kyoto* 3369–3370 (1986).
12. Reimer, L., Fromm, J. & Rennekam, R. *Ultramicroscopy* **24**, 339–354 (North-Holland, Amsterdam, 1988).
13. *Inorganic Index to the Powder Diffraction File* (Joint Committee on Powder Diffraction Standards, 1971).

ACKNOWLEDGEMENTS. We thank Drs E. Wajnberg, R. D. Machado and W. de Souza for discussions, J. Danon for advice, C. Wiedeman for pyrrhotite natural samples, Dr W. Probst for results from the CEM-902 Zeiss microscope and Drs E. X. Albuquerque and R. Broderick for the use of JEOL 1200 EX. This work was partially supported by Brazilian agencies CNPq and FINEP.

Biotransformation of ferrimagnetic greigite (Fe_3S_4) and iron pyrite (FeS_2) in a magnetotactic bacterium

Stephen Mann*, Nicholas H. C. Sparks*, Richard B. Frankel†, Dennis A. Bazylinski‡§ & Holger W. Jannasch‡

* School of Chemistry, University of Bath, Claverton Down, Bath BA2 7AY, UK

† Department of Physics, California Polytechnic State University, San Luis Obispo, California 93407, USA

‡ Department of Biology, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA

THE ability of magnetotactic bacteria to orientate and navigate along geomagnetic field lines is due to the controlled intracellular deposition of the iron oxide mineral, magnetite (Fe_3O_4)^{1,2}. The function and crystal chemical specificity of this mineral has been considered to be unique amongst the prokaryotes³. Moreover, the bacterial production of magnetite may represent a significant contribution to the natural remanent magnetism of sediments^{4,5}. Here we report, the intracellular biomineralization of single crystals of the ferrimagnetic iron sulphide, greigite (Fe_3S_4), in a multicellular magnetotactic bacterium common in brackish, sulphide-rich water and sediment. We show that these crystals are often aligned in chains and associated with single crystals of the non-magnetic mineral, iron pyrite (FeS_2). Our results have important implications for understanding biomineralization processes and magnetotaxis in micro-organisms inhabiting sulphidic environments. Furthermore, the biogenic production of magnetic iron

§ Present address: Department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, USA.

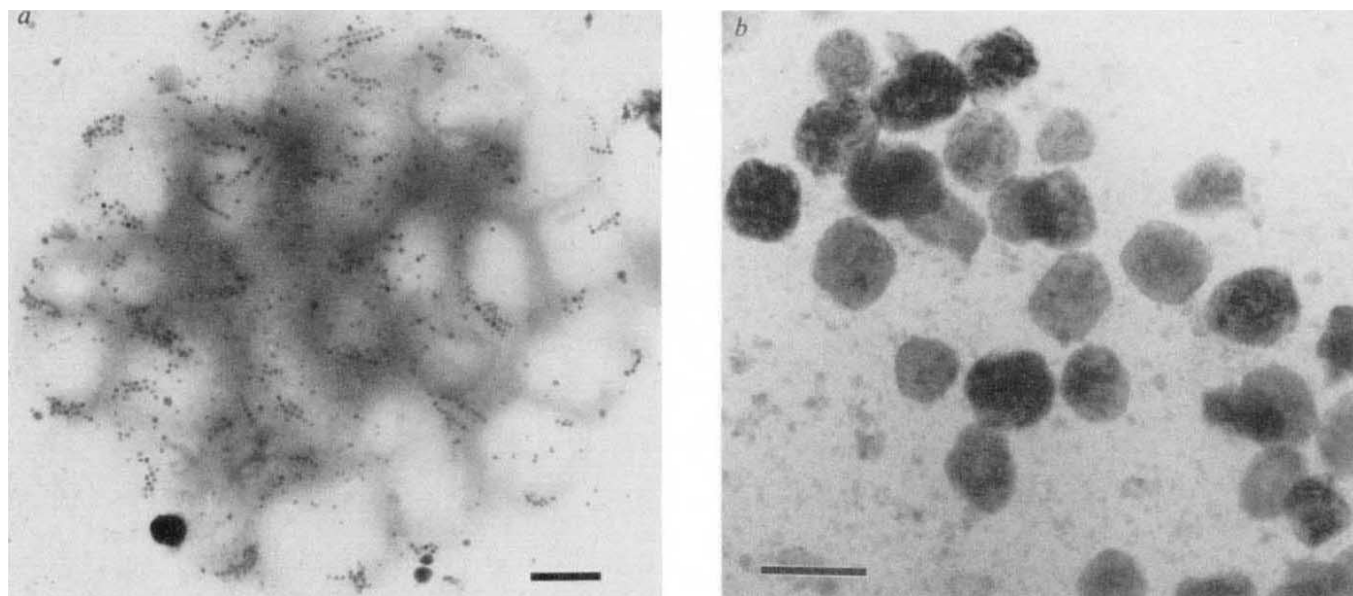


FIG. 1 *a*, Transmission electron micrograph showing a multicellular, magnetotactic bacterium containing discrete, organized, intracellular iron sulphide particles. Scale bar, 1 μm . *b*, High-magnification image showing biominerall particles. Note the well-defined edges of many of the particles and the non-uniform diffraction contrast indicating extensive surface roughness. Scale bar, 100 nm.

sulphides should be considered as a possible source of remanent magnetization in sediments.

Magnetotactic bacteria were collected from jars filled with sediment and water from several marine and brackish (12–31 p.p.t. salt) sites with high sulphide (1–2 mM). After the sediment had settled, substantial numbers of magnetotactic bacteria were collected by allowing them to accumulate at the south pole of a bar magnet placed outside the jar just above the sediment-water interface, and subsequently drawing them up with a Pasteur pipette. The predominant organism collected this way was a motile, spherical (3–8 μm diameter) multicellular aggregate of about 7–20 individual prokaryotic cells. Each cell was roughly ovoid and bore numerous flagella on one side⁶. Intact organisms eventually disaggregated into a loose clump of individual cells.

Disaggregated cells retained a permanent magnetic dipole moment as evidenced by their response to reversals of the local magnetic field direction, but were non-motile. Intact organisms were deposited in water drops on carbon-coated electron microscope grids where they partially disaggregated as the drops dried. The samples were neither fixed nor stained; nickel grids were used because the sulphide-rich salt water and sediment reacted with copper grids. Particles were studied *in situ* with disaggregated cells by scanning transmission electron microscopy (STEM), high resolution transmission electron microscopy (HRTEM), electron diffraction and energy dispersive X-ray analysis (EDXA). Electron diffraction patterns were calibrated using standard materials. Data were recorded from groups of crystals and individual particles.

Figure 1*a* shows a low-magnification image of a typical disaggregated organism. Individual cells contained chains and groups of discrete intracellular electron dense inclusions (Fig. 1*b*). On average, each chain contained 10 particles with a mean size of 75 nm. Compositional analysis showed that the particles contained Fe and S, but not O (Fig. 2). Occasionally, low levels of oxygen were detected at the edges of the particles but this was attributed to surficial oxidation of samples stored in air. These observations are similar to those of Farina *et al.*^{7,8}, who previously studied a morphologically similar magnetotactic multicellular organism containing Fe/S-rich inclusions from marine lakes in Rio de Janeiro.

Many of the particles appeared irregular in shape, but they exhibited strong diffraction contrast (Fig. 1*b*), indicating that they were crystalline. Other particles showed rhombohedral and hexagonal outlines when viewed in projection (Fig. 1*b*). These

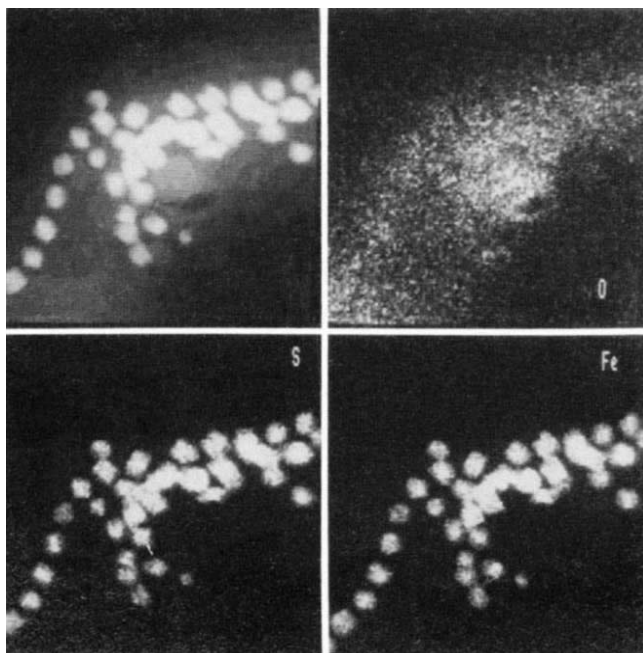


FIG. 2 Fe, S and O elemental density maps for the particles shown in the electron micrograph in the upper left part of the figure. The particles were analysed *in situ* in a magnetotactic, multicellular bacterium collected at Plum Island, Massachusetts. The maps were made by recording the respective K X-ray intensities with the energy dispersive X-ray detector at each position as the electron beam was slowly rastered across the sample. Fe and S density clearly correlate with particle position; O does not. The increased O density just below the particles correlates with increased P density (not shown), indicative of a polyphosphate granule.

images are characteristic of octahedral and cubo-octahedral particles, respectively, and indicate that the crystals could have cubic symmetry. We confirmed this by electron diffraction analysis (table 1). Powder patterns were consistent with a mixture of iron pyrite and greigite. Most of the powder patterns, however, gave d spacings corresponding to pyrite, suggesting that the magnetic mineral greigite was a minor component of the intracellular crystals. Moreover, as many of the d spacings of pyrite and greigite overlap, single-crystal diffraction patterns were required to provide unequivocal identification of the magnetic phase.

Figure 3 is a single-crystal pattern recorded from the crystal shown in the inset. The d spacings and interplanar angles (see legend for details) of this and other patterns confirmed the presence of single crystals of biogenic greigite in this organism. Other patterns corresponding to single crystals of FeS_2 were also obtained. As single-crystal diffraction patterns do not rule out the presence of amorphous domains within individual crystals, lattice imaging was undertaken. But although the crystals were in general stable under the electron beam, they were too thick for successful imaging of lattice spacings. The few lattice images recorded showed that the crystals were crystallographic single-domain particles.

The identification of intracellular, discrete, organized crystals of greigite and pyrite in magnetotactic bacteria has several important implications. It is the first conclusive evidence that prokaryotes can control the mineralization of iron sulphides. Other reports on bacterial iron sulphides⁹⁻¹¹ are concerned with anaerobic biologically mediated processes, in which sulphate-reducing bacteria generate high concentrations of H_2S which subsequently combine with iron in the extracellular environment. Minerals such as pyrite, hydrotroilite, greigite and mackinawite can be deposited. In this regard, sulphate-reducing bacteria¹¹, which can precipitate a fine-grained magnetic iron sulphide, are analogous in biomineralization terms to the anaerobic, non-magnetotactic, magnetite-producing bacterium, GS-15 (ref. 12). By contrast, the organism described here has many of the general features of biomineralization associated with genetically controlled processes. Because the environment in which it is found contains high sulphide concentrations, the organism is probably anaerobic. It is interesting that a mag-

TABLE 1 Electron diffraction data and identification of bacterial Fe sulphide inclusions

Data*	Source†	Assignment‡	
		greigite	pyrite
3.57	S	3.50 (220)	
3.16	R		3.128 (111)
3.06	S	2.98 (311)	
2.69	R		2.709 (200)
2.51	R	2.470 (400)	
2.28	S	2.26 (331)	
2.12	R		2.211 (211)
2.04	S	2.017 (422)	
1.89	R	1.901 (333)	1.915 (220)
1.71	R	1.746 (440)	
1.65	S	1.671 (531)	
1.60	R		1.633 (311)
1.55	S		1.564 (222)
1.35	R	1.383 (711)	1.354 (400)
1.26	S	1.286 (731)	
1.22	R	1.235 (800)	1.211 (420)
1.07	R	1.054 (664)	1.043 (333)
0.89	S		0.903 (600)

* Experimental d spacings in Å.

† R, powder ring pattern; S, single crystal pattern.

‡ Standard mineral d spacings in Å. X-ray powder diffraction file: greigite (16-713), and pyrite (6-710); (hkl), Miller indices.

netotactic bacterium that produces magnetite anaerobically has been isolated from the same environment¹³.

There are marked similarities between the biomineralization strategies adopted for greigite and magnetite synthesis in magnetotactic bacteria. Both systems involve the formation of discrete single crystals of restricted size that are organized within the cell. We propose that the greigite crystals, like their oxide counterparts, are enclosed within an organic membrane. Indeed, some of the crystals, when imaged at high magnification, were surrounded by an amorphous sheath, but it is possible that this arises from local contamination in the electron microscope. A major difference between the bacterial greigite and magnetite crystals is that the former have a diversity of (cubic-derived)

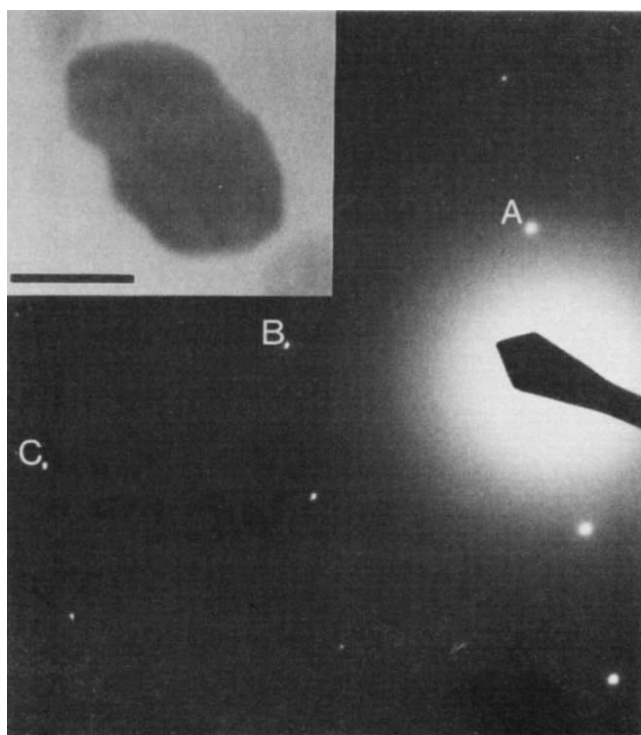


FIG. 3 Single-crystal electron diffraction pattern recorded from the crystal shown in the inset. Camera length, 284 cm; $\lambda = 0.0251$ Å; scale bar in inset, 50 nm. The pattern corresponds to the $[11\bar{3}]$ zone of greigite, Fe_3S_4 . Reflection A, $(2\bar{2}0)$ (3.50 Å); reflection B, (422) (2.02 Å); reflection C, (664) (1.054 Å). Angles: $220\wedge 422 = 73^\circ$, $220\wedge 664 = 90^\circ$. Crystallographic data: space group, $\text{Fd}\bar{3}m$ (cubic); $a = 9.876$ Å.

shapes in any one chain, whereas the latter adopt morphologies that can be unique and species-specific¹⁴. The general similarities between the two systems, however, suggests that the biological control mechanisms have much in common.

The biomineralization of bacterial magnetite involves the formation of a transient hydrous Fe(III) oxide precursor, which subsequently transforms to magnetite^{15,16}. The presence of both greigite and iron pyrite in the organism we describe indicates that there could be a similar relationship between the two mineral phases. But under thermodynamic conditions, in a highly reducing environment at neutral pH, greigite would slowly transform to pyrite¹⁷. This implies that unless the organism continues to maintain its capacity to synthesize greigite, it would slowly lose its magnetotactic response. Thus greigite may be a metastable phase in the transformation of an undetected precursor into pyrite. Alternatively, the cells might separately mineralize greigite and pyrite by local control of iron and sulphide concentration, reducing potential and pH.

Structurally, Fe₃S₄ and Fe₃O₄ are isomorphous, both materials adopting the face-centred cubic spinel structure. Whether this structural similarity has relevance to the cellular mechanisms of their synthesis is unclear. In terms of their magnetic properties, the saturation magnetization of greigite is about one-third that of magnetite¹⁸. The permanent magnetic single-domain size range for greigite has not been determined but is probably similar to magnetite, because the magnetic ordering temperatures and magnetic anisotropy constants of the two minerals are similar¹⁸. Thus, the 75-nm biogenic greigite particles are likely to be permanent magnetic single domains and responsible for the magnetotactic response of the intact organism. Organization of these particles into chains would result in a parallel alignment of the individual particle moments along the chain direction, and would give each constituent cell a permanent magnetic dipole moment equal to the sum of the individual particle moments in the chain. The net magnetic dipole moment of the intact organism would then be the vector sum of the moments of the constituent cells.

Finally, we note that greigite has been identified as a source of remanent magnetism in sediments¹⁹. These deposits may be of inorganic origin, biologically mediated by sulphate-reducing micro-organisms or resulting from the diagenesis of bacterial magnetite^{19,20}. We note, however, that the crystal size and shape of sediment greigite is similar to that reported here for bacterial greigite, and therefore propose that biogenic minerals should be considered as a further possible source of remanent magnetism in sulphide-rich sediments. □

Received 18 September; accepted 1 November 1989.

- Frankel, R. B., Blakemore, R. P. & Wolfe R. S. *Science* **203**, 1355–1356 (1979).
- Blakemore, R. P. A. *Rev. Microbiol.* **38**, 217–238 (1982).
- Lowenstam, H. A. & Weiner, S. *On Biomineralization* (Oxford University Press, 1989).
- Peterson, N., von Döbeneck, T. & Vali, H. *Nature* **320**, 611–615 (1986).
- Stolz, J. F., Chang, S.-B. R. & Kirschvink, J. L. *Nature* **321**, 849–851 (1986).
- Blakemore, R. P., Blakemore, N. A., Bazylinski, D. A. & Moench, T. T. *Bergey's Manual of Systematic Bacteriology* Vol. 3 (eds Staley, J. T. et al.) 1882–1889 (Williams and Wilkins, Baltimore, 1989).
- Farina, M., Lins de Barros, H. G. P., Esquivel, D. M. S. & Danon, J. *Biol. Cell.* **48**, 85–86 (1983).
- Farina, M., Sollarazano, G. & Viera, G. J. *Proc. Xlth Int. Cong. on Electron Microscopy (Kyoto)* 3369–3370 (1986).
- Hallberg, R. O. *Stockholm Contr. Geol.* **13**, 35–37 (1965).
- Hallberg, R. O. *N. Jahrbuch Mineral. Monatshefte* 481–500 (1972).
- Freke, M. & Tate, D. J. *biochem. microbiol. technol. Eng.* **3**, 29–39 (1961).
- Lovely, D. R., Stolz, J. F., Nord, G. L. Jr & Phillips, J. P. *Nature* **330**, 252–254 (1987).
- Bazylinski, D. A., Frankel, R. B. & Jannasch, H. W. *Nature* **334**, 518–519 (1988).
- Mann, S. & Frankel, R. B. in *Biomineralization: Chemical and Biochemical Perspectives* (eds Mann, S., Webb, J. & Williams, R. J. P.) 389–426 (VCH, Weinheim, 1989).
- Frankel, R. B., Papafthymiou, G. C., Blakemore, R. P. & O'Brien, W. D. *Biochim. biophys. Acta* **763**, 147–159 (1983).
- Mann, S., Frankel, R. B. & Blakemore, R. P. *Nature* **310**, 405–407 (1984).
- Berner, R. A. *Am. J. Sci.* **265**, 773–785 (1967).
- Sponder, M. R., Coey, J. M. D. & Morrish, A. H. *Can. J. Phys.* **50**, 2313–2326 (1972).
- Demitrack, A. in *Magnetite Biomineralization and Magnetoreception in Organisms* (eds Kirschvink, J. L., Jones, D. & MacFadden, B. J.) 625–645 (Plenum New York, 1985).
- Morse, J. W., Millero, F. J., Cornwell, J. C. & Rickard, D. *Earth Sci. Revs* **24**, 1–42 (1987).

ACKNOWLEDGEMENTS. We thank A. Garratt-Reed for assistance with the STEM measurements and R. P. Blakemore, F. G. Rogers, H. Lins de Barros and D. Esquivel for discussions. S.M. and N.H.C.S. were supported by the UK Science and Engineering Research Council. R.B.F. was supported by the US National Science Foundation. D.A.B. and H.W.J. were supported by the US National Science Foundation and the Office of Naval Research.

Mammalian sex ratios and variation in costs of rearing sons and daughters

M. Gomendio*, T. H. Clutton-Brock†, S. D. Albon†, F. E. Guinness† & M. J. Simpson*

* Sub-Department of Animal Behaviour, Madingley, Cambridge CB3 8AA, UK

† Large Animal Research Group, Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, UK

IN red deer, the sex ratio of calves at birth (calculated as the proportion of calves born that are male) increases with the dominance rank of the mother^{1,2}, whereas opposite trends exist in several populations of macaques and baboons^{3–7}. Here we show that the subsequent survival and reproductive success of subordinate female red deer is depressed more by rearing sons than by rearing daughters, whereas the subsequent fitness of dominant females is unaffected by the sex of their present offspring. By contrast, among subordinate female macaques, the rearing of daughters has greater costs to the mother's subsequent fitness than does the rearing of sons, although again, no difference in the costs of rearing sons and daughters is found among dominant mothers. These findings indicate that both differences in the relative fitness of sons and daughters and differences in the relative costs of rearing male and female offspring can favour variation in the sex ratio.

It has been suggested^{1,2} that the relationship between maternal rank and the birth sex ratio in red deer could arise because the rank of a mother exerts a stronger effect on the fitness of her sons (who compete intensely for mates) than on the fitness of her daughters. By contrast, among baboons and macaques, the rank of a female could affect the fitness of her daughters more than it affects the fitness of her sons, because daughters inherit their mother's social rank, which affects their breeding success. Sons, on the other hand, disperse to other groups where their rank and reproductive success depend extensively on alliances formed with other males^{6,7}. Here we explore the possibility that the costs of rearing sons and daughters also vary with maternal rank, and that they represent an additional selective pressure involved in the evolution of the sex-ratios which has been previously overlooked.

To determine whether the relative costs of rearing sons and daughters in ungulates and primates vary with maternal rank, we investigated the subsequent survival and reproductive performance of mothers that had raised sons versus daughters in a sample of 283 red deer hinds from the Isle of Rhum, and in a sample of 52 female macaques from the colony at Madingley, Cambridge^{5,10}. In both species, dominance rank was assessed from displacements and aggressive interactions at feeding sites^{5,11}. In red deer we used a within-cohort dominance score that removed the effects of age and scaled continuously from 0 (most subordinate) to 1 (most dominant)². In each macaque social group there were two founder females who had reproduced throughout the history of the colony, and maintained stable dominance ranks. The matriarch of the dominant family was designated as dominant, and the matriarch of the subordinate family was designated as subordinate. The other females were excluded from the analyses.

Both the survival over winter and the fecundity of adult female red deer decline with age (fitted as a quadratic function age + age²), with high rainfall in August–September, and with low winter temperature (mean daily temperature in °C in December–February¹¹). Because individuals in the population did not all breed in the same year, we have fitted terms for these phenotypic and environmental variables in generalized linear models¹² to estimate the probability of the mother's survival or fecundity in

TABLE 1 Effects of dominance and offspring sex in red deer and macaques

	Red deer						Macaques		
	χ^2	Survival d.f.	P	χ^2	Fecundity d.f.	P	χ^2	Fecundity d.f.	P
Dominance	2.0	1	NS	4.2	1	<0.05	8.3	1	<0.01
Sex of offspring	2.0	1	NS	2.2	1	NS	1.6	1	NS
Dominance-sex interaction	5.6	1	<0.02	4.0	1	<0.05	4.7	1	<0.05
Sex and dominance-sex interaction	7.6	2	<0.05	6.2	2	<0.05	6.3	2	<0.05
Dominance and sex and dominance-sex interaction	9.6	3	<0.05	10.4	3	<0.02	14.6	3	<0.01

Goodness-of-fit tests for each of the variables dominance, offspring sex and dominance-offspring sex interaction fitted in the logistic regression models (see Fig. 1) of the probability of over-winter survival and fecundity next summer in female red deer, and the probability of fecundity next year in macaques. Because the dependent variables were binary (survive, 1; die, 0; fecund, 1; not-fecund, 0) and the errors were not normally distributed least-squares regression and analysis-of-variance (ANOVA) techniques were inappropriate. Simple contingency table analysis was also inappropriate because dominance rank among deer was a metric (continuous) variable. Binary data must be related to any metric variable in a nonlinear way. The logistic regression model used here is a form of generalized linear model using a logit link function and binomial error distribution. These models permit the inclusion of significant covariates and therefore, the control of potentially confounding variables. Differences in deviance (analogous to sums of squares in ANOVA) between two models, one with and one without the test variable (for example, sex), are distributed as χ^2 with the appropriate degrees of freedom (d.f.) of the test variables. NS, not significant.

relation to her dominance rank and the sex of her offspring raised through the previous summer.

After controlling for variation due to age, August-September rainfall and winter temperature, over-winter survival in adult female deer was not related to either dominance ($\chi^2 = 2.0$, not significant) or the sex of the offspring ($\chi^2 = 2.0$, not significant), when each was added to the model separately, or together (Table 1). This was because of a significant interaction between dominance and the sex of the offspring (Table 1). Subordinate mothers that had reared male offspring showed low over-winter survival compared with dominant mothers that had reared sons (Fig. 1a). By contrast, among individuals that had produced female offspring, subordinate mothers had the same, or slightly higher, probability of survival compared with dominant mothers (Fig. 1a).

If an adult female red deer survived the winter, the probability of her calving the next summer was influenced by her dominance ($\chi^2 = 4.2$, $P < 0.05$), but not by the sex of her previous offspring ($\chi^2 = 2.2$, not significant). But as with the analysis of survival, there was a significant interaction between dominance and sex (Table 1): subordinate mothers were less likely to calve again after rearing a male offspring the previous summer than they were after rearing a female offspring. Dominant mothers were more likely to calve again than were subordinate mothers, but

the sex of the previous calf seemed to have little influence (Fig. 1b). The extra fitness costs of rearing sons versus daughters seem to be substantial among subordinate mothers but absent among dominant mothers.

Contrasting differences were found in macaques. Subordinate mothers were less likely to breed again the next year than were dominant mothers ($\chi^2 = 8.3$, $P < 0.01$). The interaction with sex (Table 1) was reversed: subordinate mothers were more likely to give birth the following season after rearing a male offspring than they were after rearing a female offspring, whereas dominant mothers were slightly more likely to give birth the following season after rearing a female offspring than after rearing a male offspring (Table 2).

The mechanisms responsible for interactions between maternal rank and the costs of rearing sons and daughters may be

FIG. 1 Logistic curves illustrating the probability of female red deer surviving a winter (a) and calving in the year after rearing a son versus a daughter (b), plotted against their dominance score. Logistic regressions¹³ were of the form:

$$\text{Probability } (Y_1 = 1) = \frac{\exp G(X_1)}{1 + \exp G(X_1)}$$

where $G(X_1) = (A + B_1 x_{11} + B_2 x_{12} + B_3 x_{13} + \dots + B_7 x_{17})$ and $A, B_1, B_2, \dots, B_7$ are constants, x_{11} is dominance rank, x_{12} is offspring sex, x_{13} is dominance-sex interaction, x_{14} is age, x_{15} is age², x_{16} is August-September rainfall (mm), x_{17} is mean daily temperature (°C) December-February, and $i = 1, 2, 3, \dots, N$ hind-years. Curves in (a) are standardized for age (12 years), August-September rainfall (750 mm) and December-February mean daily temperature (4 °C). Coefficients in the exponential equation (see above) are—males: $B_1 = 1.573$, $B_2 = 0$, $B_3 = 0$; females: $B_1 = 1.573$, $B_2 = 1.615$, $B_3 = -2.236$; both sexes have the same values for coefficients A (0.73), B_4 (0.383), B_5 (-0.0347), B_6 (-0.003572), and B_7 (0.504). Curves in (b) are standardized for age (12 years), August-September rainfall (750 mm), and December-February mean daily temperature (4 °C). Coefficients in the exponential equation (see above) are—males: $B_1 = 0.526$, $B_2 = 0$, $B_3 = 0$; females: $B_1 = 0.526$, $B_2 = 0.476$, $B_3 = -0.580$; both sexes have the same values for coefficients A (-5.070), B_4 (0.916), B_5 (-0.04746), B_6 (-0.003087), and B_7 (0.5681).

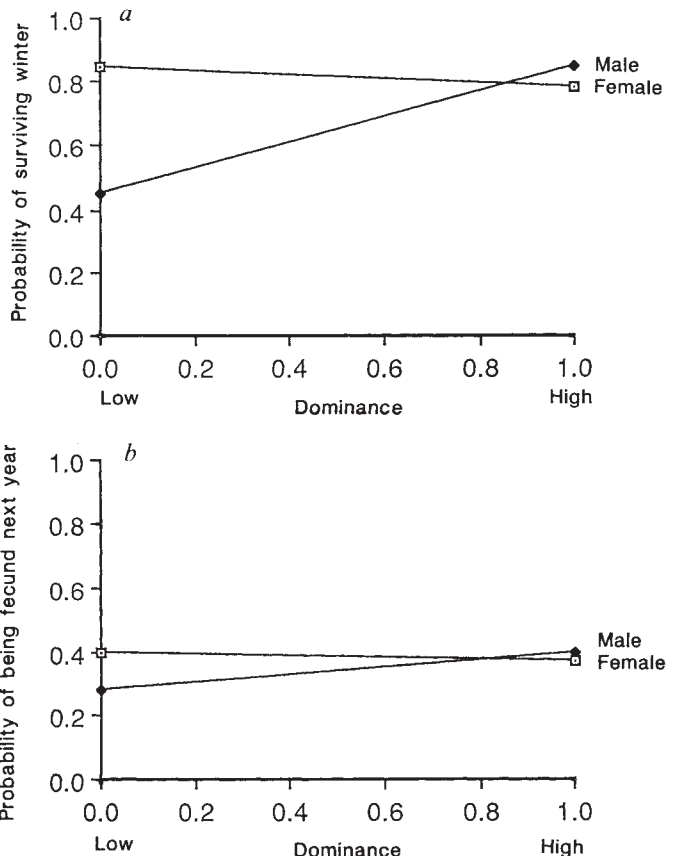


TABLE 2 Probability of fecundity in macaques

	Male reared	Female reared
Mother's rank		
Dominant	0.80 (5)	0.92 (14)
Subordinate	0.62 (8)	0.00 (4)

Probability of subordinate and dominant macaques giving birth the year after rearing sons versus daughters. Number of births sampled are given in parentheses. The generalized linear model for macaques only incorporated the variables dominance, sex, and dominance-sex interaction (see Table 1).

quite different in ungulates and primates. In red deer, goats, and bison, as well as in African elephants^{9,14,15}, sons are suckled more frequently than are daughters during the peak period of lactation and the energetic costs of rearing males probably exceed those of rearing females⁹. Subordinate females could be more strongly affected by these differences because they do not have priority of access to the best feeding sites, and because their body condition is generally poorer than that of dominant females¹⁰. By contrast, no overall differences in suckling were found between male and female infants in rhesus macaques. But, when the interaction between infant sex and maternal rank was examined, it was found that daughters of subordinate mothers tended to be suckled more frequently than their sons, and more than the infants of dominant mothers⁵. Allowing frequent access to the nipple could have been a maternal response to the high levels of aggression and harassment that the daughters of subordinate mothers tended to receive from unrelated females, a phenomenon which has been documented in several studies^{7,16}. Frequent nipple stimulation inhibits ovulation in mammals¹⁷, and this could have been responsible for the longer delays before the next conception among subordinate females that had reared daughters^{5,18}.

Consequently, the results presented here indicate that two separate selection pressures can favour the evolution of the contrasting sex-ratios in ungulates^{1,19-21} and cercopithecine primates³⁻⁷. First, maternal rank can have opposite effects on the relative fitness of sons and daughters^{1,2,6,7}. Second, maternal rank can modify the relative costs of rearing sons and daughters, favouring the production of daughters by subordinate mothers in red deer, and the production of sons by subordinate mothers in macaques. In both cases, these biases could favour the production of an excess of the other sex by dominant females²². □

Received 20 July; accepted 27 November 1989.

- Clutton-Brock, T. H., Albon, S. D. & Guinness, F. E. *Nature* **308**, 358-360 (1984).
- Clutton-Brock, T. H., Albon, S. D. & Guinness, F. E. *Anim. Behav.* **34**, 460-461 (1986).
- Simpson, M. J. A., Simpson, A. E., Hookey, J. & Zunz, M. *Nature* **290**, 49-51 (1981).
- Simpson, M. J. A. & Simpson, A. E. *Nature* **300**, 440-441 (1982).
- Gomendio, M. thesis, Univ. Cambridge (1988).
- Altmann, J. *Baboon Mothers and Infants* (Harvard University Press, 1980).
- Silk, J. B. *Am. Nat.* **121**, 56-66 (1983).
- Ritchie, M. *Am. Nat.* (in the press).
- Clutton-Brock, T. H., Albon, S. D. & Guinness, F. E. *Nature* **289**, 487-489 (1981).
- Clutton-Brock, T. H., Guinness, F. E. & Albon, S. D. *Red Deer: Behaviour and Ecology of Two Sexes* (University of Chicago Press, 1982).
- Clutton-Brock, T. H., Albon, S. D. & Guinness, F. E. *Nature* **337**, 260-262 (1989).
- McCullough, P. & Nelder, J. A. *Generalized Linear Models* (Chapman & Hall, London, 1983).
- Cox, D. R. *The Analysis of Binary Data* (Methuen, London, 1970).
- Pickering, S. P. C. thesis, Univ. Durham (1983).
- Lee, P. C. & Moss, C. J. *Behav. Ecol. Sociobiol.* **18**, 353-361 (1986).
- Dittus, W. P. J. *Behaviour* **69**, 265-302 (1979).
- McNeilly, A. S. in *The Physiology of Reproduction* (eds Knobil, E. & Neill, J.) 2323-2349 (Raven, New York, 1988).
- Gomendio, M. *J. Zool.* **217**, 449-467 (1989).
- Wolff, J. O. *Behav. Ecol. Sociobiol.* **23**, 127-133 (1988).
- Dhillon, J. S., Acharya, R. M., Tiwana, M. S. & Aggarwal, S. C. *Anim. Prod.* **12**, 81-87 (1970).
- Singh, O. N., Singh, R. N. & Srivastava, R. R. P. *Ind. J. vet. Sci.* **35**, 245-248 (1965).
- Fisher, R. A. *The Genetical Theory of Natural Selection* (Oxford University Press, 1930).

ACKNOWLEDGEMENTS. We thank Robert Hinde and Pat Bateson for providing facilities at Madingley, the MRC for access to the macaque colony, the director of the Nature Conservancy Council (Scotland) and the staff of the NCC for permission to work on Rhum, and all those who have helped on the Rhum red deer project for assistance with collecting data on reproductive success and survival. The research was funded by NERC, SERC, the Royal Society, St John's College and Trinity Hall.

A network that learns to recognize three-dimensional objects

T. Poggio & S. Edelman

Artificial Intelligence Laboratory, Center for Biological Information Processing, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

THE visual recognition of three-dimensional (3-D) objects on the basis of their shape poses at least two difficult problems. First, there is the problem of variable illumination, which can be addressed by working with relatively stable features such as intensity edges rather than the raw intensity images^{1,2}. Second, there is the problem of the initially unknown pose of the object relative to the viewer. In one approach to this problem, a hypothesis is first made about the viewpoint, then the appearance of a model object from such a viewpoint is computed and compared with the actual image³⁻⁷. Such recognition schemes generally employ 3-D models of objects, but the automatic learning of 3-D models is itself a difficult problem^{8,9}. To address this problem in computational vision, we have developed a scheme, based on the theory of approximation of multivariate functions, that learns from a small set of perspective views a function mapping any viewpoint to a standard view. A network equivalent to this scheme will thus 'recognize' the object on which it was trained from any viewpoint.

Is the need for 3-D range-based or manually specified models real? Structure from motion theorems^{10,11}, pioneered by Ullman¹², indicate that full information about the 3-D structure of an object represented as a set of feature points (at least five to eight) is present in just two of their perspective views, provided that corresponding points are identified in each view. A view is represented as a $2N$ vector $x_1, y_1, x_2, y_2, \dots, x_N, y_N$ of the coordinates on the image plane of N labelled and visible feature points on the object. Here, and in most of the following, we assume that all features are visible, as they are in wire-frame objects. The generalization to opaque objects follows by partitioning the viewpoint space for each object into a set of 'aspects'¹³, corresponding to stable clusters of visible features. In principle, therefore, having enough 2-D views of an object is equivalent to having its 3-D structure specified.

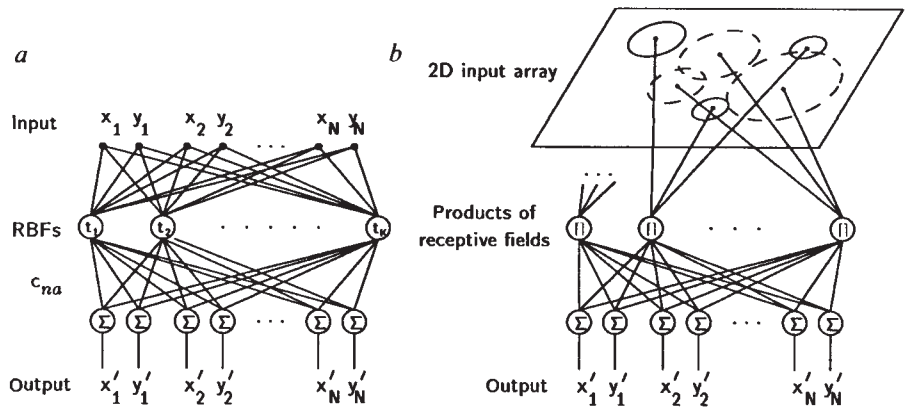
This line of reasoning, together with properties of perspective projection, indicate (1) that for each object there exists a smooth function mapping any perspective view into a 'standard' view of the object, and (2) that this multivariate function can be synthesized, or at least approximated, from a small number of views of the object. Such a function would be object-specific, with different functions corresponding to different 3-D objects. Furthermore, the application of the function that is specific for one object to the views of a different object is expected to result in a 'wrong' standard view that can be easily detected as such.

Synthesizing an approximation to a function from a small number of sparse data—the views—can be considered as learning an input-output mapping from a set of examples^{14,15}. A powerful scheme for the approximation of smooth functions has been recently proposed under the name of Generalized Radial Basis Functions (GRBFs), and shown^{14,15} to be equivalent to standard regularization^{16,17} and generalized splines (ref. 14; see closely related work by Powell¹⁸, and Broomhead and Lowe¹⁹). The approximation of $f: R^n \rightarrow R$ is given by

$$f(\mathbf{x}) = \sum_{\alpha=1}^K c_{\alpha} G(\|\mathbf{x} - \mathbf{t}_{\alpha}\|) \quad (1)$$

where the K coefficients c_{α} and the centres $\mathbf{t}_{\alpha}$ are found during the learning stage and G is an appropriate basis function (see refs 14 and 15), such as the gaussian function. A polynomial term of the form $\sum_i d_i p_i(\mathbf{x})$ can be added to the right-hand side of equation (1). In this paper we omit the polynomial term (see

FIG. 1 *a*, Network representation of approximation by GRBFs. In a special simple case, there are as many basis functions (K) as views in the training set (M ; in general, $K \leq M$). The centres of the radial functions are then fixed and are identical with the training views. Each basis unit in the 'hidden' layer computes the distance of the new view from its centre and applies to it the radial function. The resulting value $G(\|x - t_a\|)$ can be regarded as the 'activity' of the unit. If the function G is gaussian, a basis unit will attain maximum activity when the input exactly matches its centre. The output of the network is the linear superposition of the activities of all the basis units in the network. *b*, An equivalent interpretation of *a* for the case of gaussian radial basis functions. A multidimensional gaussian function can be synthesized as the product of 2-D gaussian receptive fields operating on retinotopic maps of features. The solid circles in the image plane represent the 2-D gaussian functions associated with the first radial basis function, which corresponds to the first view of the object. The dotted circles represent the 2-D receptive fields that synthesize the gaussian



radial function associated with another view. The gaussian receptive fields transduce positions of features represented implicitly as activity in a retinotopic array, and their product 'computes' the radial function without the need of calculating norms and exponentials explicitly.

ref. 14). If the function f is vector-valued, each component f_i is computed using equation (1) with the appropriate c_{ia} , in which case the equation is equivalent to the network of Fig. 1.

The weights c are found during learning by minimizing a measure of the error between the network's prediction and the desired output for each of the M examples. Computationally, this amounts to inverting a matrix (when $M \neq K$, the generalized inverse is computed instead). When the number of basis functions is less than the number of views in the training set, the centres of the basis functions are also updated during learning. Updating the centres is equivalent to modifying the corresponding 'prototypical views'. For a detailed description of this approximation technique, of its theoretical motivation and its relation to other techniques such as backpropagation²⁰, see refs 14 and 15.

Figure 2 shows an application of GRBFs to the recognition problem. We consider here the special case of recognizing a wire-frame 3-D object from any of its perspective views with N feature points (we mainly used $N = 6$). A GRBF module, trained on several tens of random views, maps any new view of the same object into a standard view (for example, into one of the initially chosen training views).

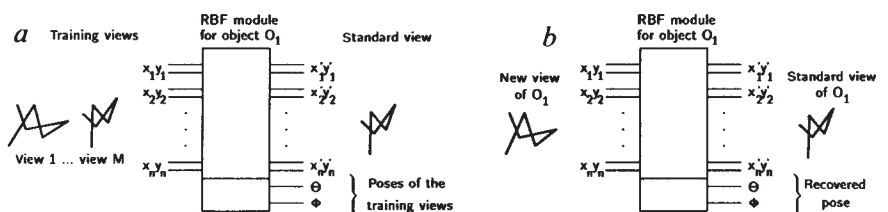
We have also explored the use of fewer basis functions than training views and used gradient descent to look for the optimal locations of the centres t_a in addition to the optimal value of c_a . We found satisfactory performance with just two basis units (for 10–40 training views and with the attitude of the object limited to one octant of the viewing sphere). This indicates that a very small number of units are needed for each aspect¹³ of an opaque object (compare with ref. 21). It is of interest that after training, the centres of the radial basis units correspond to views that are different from any of the training views.

It should be clear that the scheme proposed here addresses

only one part of the problem of shape-based object recognition, the variability of object appearance due to changing viewpoint. The key issue of how to detect and identify image features that are stable for different illuminations and viewpoints is outside the scope of this paper. Notice that the GRBF approach to recognition does not require the x, y coordinates of image features as inputs: other parameters of appropriate features could also be used, such as a corner angles (see Fig. 4a) or segment lengths (compare ref. 4 and M. Villalba, thesis in preparation), or the colour and the texture of the object. Recognition of noisy and partially occluded objects, using realistic feature identification schemes, requires an extension of the scheme, even if the problems of object segmentation and selection²² are addressed separately. A natural extension of the scheme could be based, for example, on the use of multiple lower-dimensional centres, corresponding to different subsets of detected features, instead of one $2N$ -dimensional centre for each view in the example set. Our initial experiments²³ support the notion that a scheme based on low-dimensional centres is useful for recognition while being robust against occlusions and noise. Another possible extension of the scheme involves a hierarchical composition of GRBF modules, in which the outputs of lower-level modules assigned to detect objects parts and their relative disposition in space are combined to allow recognition of complex-structured objects.

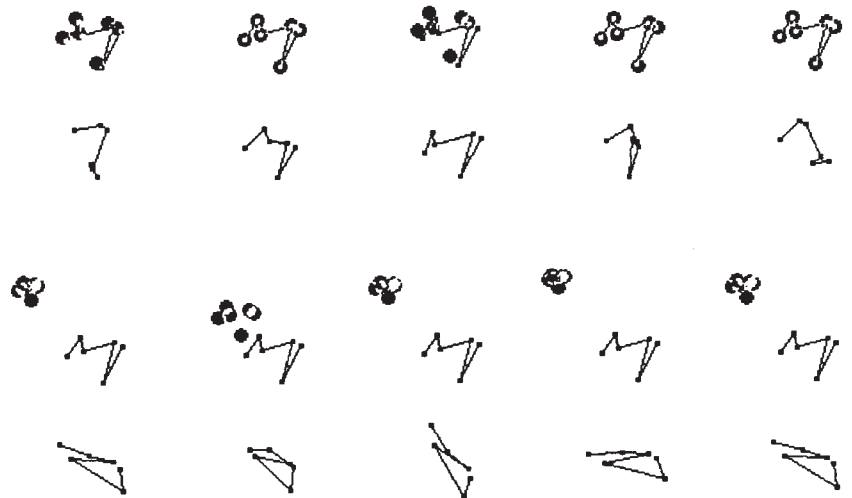
In a sense, the application of the GRBF method to recognition can be considered as a generalization of the exact approach of Basri and Ullman²⁴. They have recently shown that under orthographic projection, any view of a 3-D object undergoing a linear group of transformations that includes rigid transformation in 3-D space (that is, translations and rotations) can be obtained from three fixed views. They used this result to synthesize a linear operator that, for orthographic projection, maps exactly

FIG. 2 Application of a general module for multivariate function approximation to the problem of recognizing a 3-D object from any of its perspective views. *a*, Module is trained to produce the vector representing the standard view of the object, given a set of examples of random perspective views of the same object. The module is also capable of recovering the viewpoint coordinates θ, ϕ (the latitude and the longitude of the camera on an imaginary sphere centred at the object) that correspond to the training views. When given a new random view of the same object (*b*), the module recognizes it by producing the standard view. Other objects are rejected by



thresholding the euclidean distance between the actual output of the model and the standard view (this step corresponds to the action of a single radial function with a sharp cut-off centred on the standard view).

FIG. 3 Some examples of the module's operation. Standard view of a wire-frame object (top row) superimposed on its estimate by the GRBF network (large dots) when its input is a random view of the same object (second row from top). The fit is much closer than in the bottom two rows, where the input view belongs to a different object. The number of training views M is 40, the number of RBFs K is 20, and the range of attitudes θ, ϕ is 0° – 90° . Gradient descent was used to obtain the optimal positions of the GRBF centres. Within a smaller range of $\theta, \phi \in [0^\circ, 45^\circ]$, the performance was acceptable with only two radial basis units ($M=40, K=2$).



each view of a given object into the zero vector and performs fairly well also for most cases of perspective projection²⁴. By comparison, the GRBF approach is based on an approximation, even in the orthographic case, and typically needs more than three views. But it can (1) use as inputs feature parameters other than the x, y coordinates (Fig. 4a) and (2) recover parameters, such as the attitude angles of the input object (Fig. 4d), that do not depend linearly on the views of the object.

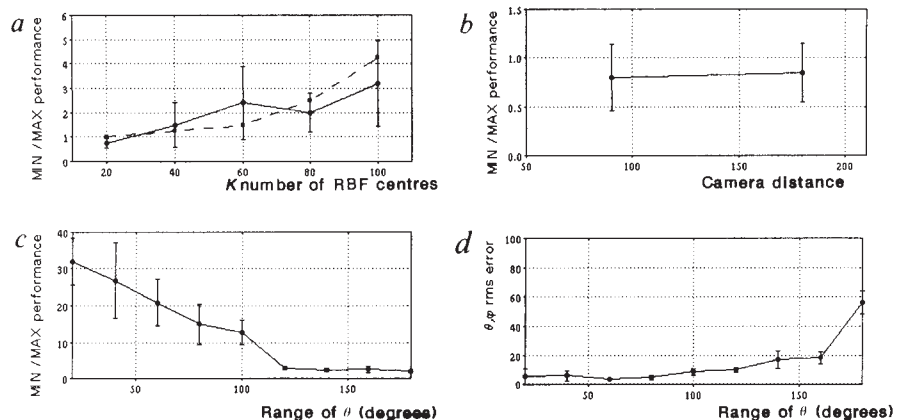
In some respects, the performance of the GRBF-based recognition scheme resembles human performance in a related task. For example, the number of training views necessary to achieve an acceptable recognition rate on novel views, 80–100 for the full viewing sphere, is broadly compatible with the finding²⁵ that people have trouble recognizing a novel wire-frame object previously seen from one viewpoint if it is rotated away from that viewpoint by about 30° (it takes $72\ 30^\circ \times 30^\circ$ -patches to cover the viewing sphere). Furthermore, a network model recently shown to capture some of the time-course and learning characteristics of the recognition process²⁶, seems to be computationally related to GRBFs²⁷. Experiments designed to test specific predictions of GRBF and several other recognition schemes^{24,27} are now under way in our laboratory.

One feature of the GRBF scheme that could guide its interpre-

tation in biological terms is the possibility of decomposing a multidimensional gaussian radial basis function into a product of gaussian functions of lower dimensions (Fig. 1b). In our case, the centre of a basis unit is similar to a prototype and the unit itself is synthesized as the product of feature detectors with 2-D gaussian receptive fields (that is, the activity of a detector depends on the distance r between the stimulus and the centre of the receptive field as e^{-r^2/σ^2}). The network's output (see equation 1) is the sum of products and therefore represents the logical disjunction of conjunctions ' $\vee_\alpha \wedge_i$ (feature F_i at (x_i, y_i))', where the disjunction ranges over all the prototypes of the given object.

The adjustment of weights c_α in the GRBF network in Fig. 1 through some pseudo-hebbian mechanism is not biologically implausible. Alternatively, a plausible biophysical implementation of the gradient-descent update of the centres (or, as in Fig. 1b, the location of the receptive fields) is problematic. But notice that reasonable initial performance can be obtained merely by setting the centres to a subset of the examples. A subsequent possibly slow process, much simpler and more plausible than gradient descent, may then search for optimal positions. Another possible solution is to select for each object a set of optimally located receptive fields out of a large available population^{27,14}.

FIG. 4 *a*, Performance of a GRBF module trained to recognize a specific object over the full range of θ, ϕ (the entire viewing sphere). Views were encoded as vectors of $2N$ vertex coordinates (solid curve; error bars show the s.d. of the performance indices, computed over a set of 10 objects, each of which served in turn as the target) or as vectors of $N-2$ angles formed by pairs of segments (dashed curve). In these examples, the number of training views M is chosen to equal the number of radial basis functions K . The performance index MIN/MAX is defined as the ratio of the smallest euclidean distance E obtained for views of different objects to the largest E obtained over a set of novel random views of the object on which the module has been trained. MIN/MAX > 1 is required for a perfect separation between the target and other objects using a simple threshold decision. For nearly perfect recognition, 80–100 views suffice. *b*, Performance for two conditions—near and far—corresponding to relatively high and low perspective distortion, respectively (full range of θ, ϕ in both cases). *c*, GRBF shows a slow degradation in performance with increasing range of the viewpoint coordinates θ, ϕ (the objects are a cube and an octahedron, $M=K=40$, and the error bars are



s.d. over 10 sets of random training and testing views). *d*, GRBF can also provide a good estimate of the attitude of the object. The inset shows the errors in the viewpoint coordinates θ, ϕ recovered by the module versus the range of the viewpoint coordinates. In *c* and *d*, $\phi_{\max} = 2\theta_{\max}$, so that $\theta_{\max} = 180^\circ$ corresponds to the full viewing sphere.

Sensory-input driven selection of representation units has been demonstrated *in vivo* (for example, see refs 28 and 29).

The GRBF recognition scheme seems reasonable in terms of the biophysical mechanisms required, is attractive because an effective computation is simply performed by the combination of receptive fields, and is surprising because it bases a scheme involving units somewhat similar to 'grandmother' cells (compare refs 30 and 31) on the rigorous approximation methods of regularization and splines. □

Received 9 August; accepted 20 November 1989.

- Marr, D. *Vision* (Freeman, San Francisco, 1982).
- Poggio, T., Gamble, E. B. & Little, J. J. *Science* **242**, 436–440 (1988).
- Fischler, M. A. & Bolles, R. C. *Commun. ACM* **24**, 381–395 (1981).
- Thompson, D. W. & Munday, J. L. in *Proc. IEEE Conf. Robotics and Automation* 208–220 (Raleigh, North Carolina, 1987).
- Huttenlocher, D. P. & Ullman, S. in *Proc. 1st Int. Conf. Computer Vision* 102–111 (IEEE, Washington DC, 1987).
- Lowe, D. G. *Perceptual Organization and Visual Recognition* (Kluwer Academic Publishers, Boston, Massachusetts, 1986).
- Ullman, S. *Cognition* **32**, 193–254 (1989).
- Grimson, W. E. L. & Lozano-Pérez, T. *IEEE Trans. Pattern Analysis Machine Intell.* **9**, 469–482 (1987).
- Fan, T. J., Medioni, G. & Nevatia, R. in *Proc. 2nd Int. Conf. Computer Vision* 474–481 (Florida, IEEE, Washington DC, 1988).
- Tsai, R. Y. & Huang, T. S. *IEEE Trans. Pattern Analysis Machine Intell.* **6**, 13–27 (1984).
- Longuet-Higgins, H. C. *Nature* **293**, 133–135 (1981).
- Ullman, S. *The Interpretation of Visual Motion* (MIT Press, Cambridge, Massachusetts, 1979).
- Koenderink, J. J. & van Doorn, A. J. *Biol. Cybern.* **32**, 211–217 (1979).

- Poggio, T. & Girosi, F. *Artif. Intell. Lab. Memo No. 1,140* (Artificial Intelligence Laboratory, MIT, Cambridge, 1989).
- Poggio, T. & Girosi, F. *Science* (in the press).
- Tikhonov, A. N. & Arsenin, V. Y. *Solutions of Ill-posed Problems* (Winston, Washington DC, 1977).
- Poggio, T., Torre, V. & Koch, C. *Nature* **317**, 314–319 (1985).
- Powell, M. J. D. in *Algorithms for Approximation* (eds Mason, J. C. & Cox, M. G.) (Clarendon, Oxford, 1987).
- Broomhead, D. S. & Lowe, D. *Complex Syst.* **2**, 321–355 (1988).
- Rumelhart, D. E., Hinton, G. E. & Williams, R. J. *Nature* **323**, 533–536 (1986).
- Perrett, D. I., Mistlin, A. J. & Chitty, A. J. *Trends Neurosci.* **10**, 358–364 (1989).
- Edelman, S. & Poggio, T. *Optic News* **15**, 8–15, May 1989.
- Poggio, T. & Edelman, S. *Artif. Intell. Lab. Memo No. 1,181* (Artificial Intelligence Laboratory, MIT, Cambridge, 1989).
- Basri, R. & Ullman, S. *Artif. Intell. Lab. Memo No. 1,152* (Artificial Intelligence Laboratory, MIT, Cambridge, 1989).
- Rock, I. & DiVita, J. *Cognitive Psychol.* **19**, 280–293 (1987).
- Edelman, S., Bülthoff, H. & Weinshall, D. *Artif. Intell. Lab. Memo No. 1,138* (Artificial Intelligence Laboratory, MIT, Cambridge, 1989).
- Edelman, S. & Weinshall, D. *Artif. Intell. Lab. Memo No. 1,146* (Artificial Intelligence Laboratory, MIT, Cambridge, 1989).
- Jenkins, W. M., Merzenich, M. M. & Ochs, M. T. *Soc. Neurosci. Abstr.* **10**, 665 (1984).
- Edelman, G. M. & Finkel, L. in *Dynamical Aspects of Neocortical Function* (eds Edelman, G. M., Gall, W. E. & Cowan, W. M.) 653–695 (Wiley, New York, 1984).
- Gross, C. G., Rocha-Miranda, C. E. & Bender, D. B. *J. Neurophys.* **35**, 96–111 (1972).
- Perrett, D. I., Rolls, E. T. & Caan, W. *Expl Brain Res.* **47**, 329–342 (1982).

ACKNOWLEDGEMENTS. We thank F. Crick, F. Girosi, E. Grimson, E. Hildreth, D. Hillis, A. Hurlbert, L. Tucker, S. Ullman and D. Weinshall for discussion. The work was done in the Artificial Intelligence Laboratory and the Center for Biological Information Processing in the Department of Brain and Cognitive Sciences. This research was supported by the ONR, Cognitive and Neural Sciences Division, and the Artificial Intelligence Center of Hughes Aircraft Corporation. Support for the A.I. Laboratory's artificial intelligence research is provided by the Advanced Research Projects Agency of the Department of Defense. T.P. is supported by the Uncas and Helen Whitaker chair. S.E. is supported by a Chaim Weizmann Postdoctoral Fellowship from the Weizmann Institute of Science.

A second molecular form of D₂ dopamine receptor in rat and bovine caudate nucleus

Christopher L. Chio*, Gerard F. Hess†, R. Scott Graham† & Rita M. Huff*

* Cell Biology and † Molecular Biology, The Upjohn Co., Kalamazoo, Michigan 49001, USA

OVEREXPRESSION of the D₂ dopamine receptor has been proposed to be part of the pathology of schizophrenia¹. The isolation of a D₂ dopamine receptor clone has assisted the molecular characterization of D₂ receptors². We have now isolated an identical rat clone along with two other clones—a second related rat clone (RD-2_{in}) and a homologous bovine clone (BD-2_{in}), both of which contain an insert encoding an additional 29 amino acids relative to the original rat clone (RD-2_o). All three clones encode D₂ receptor binding sites when expressed in COS-7 cells. The amino-acid insert encoded by D-2_{in} lies in the domain of the receptor believed to interact with the GTP-binding proteins (G proteins) of various signal transduction pathways³. By using oligonucleotide probes specific for either D-2_o or D-2_{in} RNA transcripts, we have found that the level of expression of the D-2_{in}-encoded form of the receptor is seven times that of the D-2_o form in the caudate nucleus, the richest brain source of D₂ receptors⁴.

We have isolated a clone, RD-2_o, that was shown by sequencing to be identical (between the *Xho*I and *Pst*I sites) to the rat D₂ clone of Bunzow *et al.*² We labelled RD-2_o complementary DNA with random primer and used it as a hybridization probe to screen a bovine caudate nucleus cDNA library. We identified and isolated a single positive plaque, and found the cloned DNA fragment to comprise 2,376 bases. The DNA sequence and the amino-acid sequence of the longest open reading frame are shown in Fig. 1. Alignment of the bovine clone with RD-2_o showed that the two clones are highly homologous, except that the bovine clone contains an insert of 87 nucleotides that is not found in RD-2_o. Thus, the bovine clone encodes a protein of 444 amino acids compared with the 415 amino acids encoded

by RD-2_o. Excluding the insert, the clones from the two species are 85% identical at the nucleotide sequence level and 96% identical at the amino-acid sequence level, with the few amino-acid differences being conservative changes.

Further screening of the original rat brain cDNA library revealed that of 16 positive clones, 4 had cloned DNA fragments of ~1,450 bases (one of these is RD-2_o), and 12 had cloned DNA fragments of ~1,550 bases. DNA from a clone of ~1,550 bases, clone RD-2_{in}, was sequenced and found to be identical to RD-2_o, including the noncoding sequence between the *Xho*I and *Pst*I sites, except for an additional 87 nucleotides inserted between nucleotides 723 and 724 of RD-2_o. The additional sequence in RD-2_{in} is inserted at the same location and is highly similar (97%) to the bovine insert sequence.

D₂ dopamine receptors belong to a family of receptors whose members have in common an interaction with one of the G proteins for signal transduction. All family members sequenced so far have structural similarities, including seven putative transmembrane domains and a putative G protein-coupling domain in the third intracytoplasmic loop⁵. It is in the putative third intracytoplasmic loop of the D₂ dopamine receptor that the additional coding sequences of RD-2_{in} and BD-2_{in} are found. The amino-acid sequences encoded by the two rat clones and the bovine clone flanking and including the insert region are shown in alignment in Fig. 2. The amino-acid sequence encoded by the insert in RD-2_{in} is identical to that encoded by the insert in BD-2_{in}, except for a single methionine-to-valine substitution.

We transfected all three clones into African green monkey kidney cells (COS-7) and measured D₂ binding sites using the D₂ dopamine receptor antagonist [³H]spiroperidol. We detected high and equivalent levels of D₂ receptors in homogenates of COS-7 cells transfected with each clone (Fig. 3). Furthermore, the newly expressed receptors had similarly high affinities for [³H]spiroperidol (*K*_d = 18 pM (RD-2_o); *K*_d = 22 pM (RD-2_{in}); *K*_d = 24 pM (BD-2_{in}); *K*_d, dissociation constant)—the same as the affinity of the D₂ receptor in the caudate nucleus for spiroperidol and 1,000 times the affinity of the D₁ dopamine receptor for spiroperidol⁶. The affinities of RD-2_o, RD-2_{in} and BD-2_{in}-encoded receptors for dopamine in membranes prepared from transfected cells were also nearly identical, at ~1–2 μM.

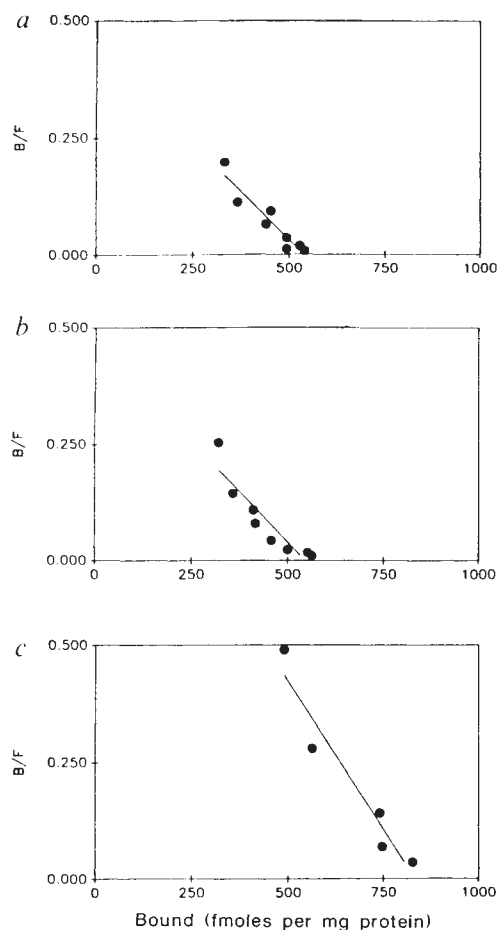
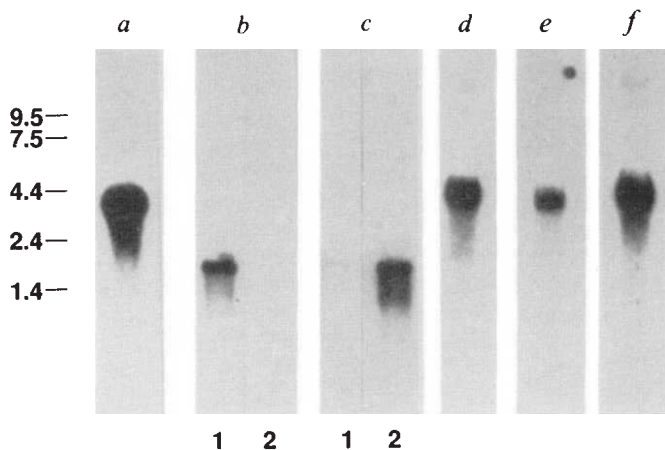


FIG. 3 Scatchard plots of the binding of [3 H]spiroperidol to homogenates of COS-7 cells transfected with RD-2_o, RD-2_{in} or BD-2_{in}. Saturation binding curves of specific [3 H]spiroperidol binding were transformed by the method of Scatchard¹⁴. *a*, Binding of [3 H]spiroperidol to homogenates of COS-7 cells transfected with RD-2_o. The maximum bound ligand (B_{max}) is 540 fmol per mg protein and the K_d is 18 pM. *b*, Binding of [3 H]spiroperidol to homogenates of COS-7 cells transfected with RD-2_{in}. B_{max} , 540 fmol per mg protein; K_d , 22 pM. *c*, Binding of [3 H]spiroperidol to homogenates of COS-7 cells transfected with BD-2_{in}. B_{max} , 830 fmol per mg protein; K_d , 24 pM. Less than 50 fmol per mg protein [3 H]spiroperidol binding sites were detected in membranes of mock-transfected cells.

METHODS. RD-2_o, RD-2_{in} and BD-2_{in} clones were inserted as *Xho*I-*Xba*I fragments into the SV40 late promoter expression vector plasmid pSVL (Pharmacia). One day before transfection, 100-mm dishes (Falcon) were seeded with African green monkey kidney COS-7 cells (ATCC-cRL 1651), at a density of 2.5×10^5 cells per dish. Purified plasmid (5 μ g) was used to transfect a COS-7 monolayer per dish. DEAE-dextran (Sigma) was used to facilitate transfection (ref. 15, as modified in ref. 16). After infection (48h) cells were collected for receptor-binding assays by scraping into 5 ml PBS. Homogenates were prepared by dounce homogenization in 50 mM Tris buffer, pH 7.4, 6 mM MgCl₂ and then freeze-thawed in liquid nitrogen. The assays were carried out in a volume of 1 ml containing 20–30 μ g protein, varying concentrations of [3 H]spiroperidol (10–500 pM), and 0.1% ascorbic acid, 0.1% BSA vehicle (for total binding) or 1 μ M (+) butaclamol (for nonspecific binding). Incubations were carried out at 30 °C for 1 h and terminated by addition of 4 ml ice-cold wash buffer (10 mM Tris buffer, pH 7.4), filtration over glass-fibre filters (Whatman GF/C) followed by 2×10 ml wash buffer. Filters were counted by liquid scintillation spectroscopy.

FIG. 4 Northern blot analysis of D-2_{in} and D-2_o dopamine receptor RNA transcripts. Autoradiographs of northern blots of various RNA samples hybridized with D₂-specific oligonucleotide or cDNA probes are shown. To the left of the blots is the size (in kb) as determined from RNA size markers (BRL). *a*, Random-primer-labelled RD-2_o cDNA hybridized with a 3.2-kb band of rat caudate nucleus poly(A)⁺ RNA. Messenger RNA (5 μ g) was loaded. The autoradiograph was exposed for 3 h. *b*, Complementary RNA (20 ng) synthesized from the RD-2_{in} clone (lane 1), or 20 ng cRNA synthesized from the RD-2_o clone (lane 2), hybridized with the insert oligonucleotide probe (5'-GCCTGTCTCACTGGGAAATCCCAT-3'). The blot was exposed to film for 8 h. *c*, RD-2_{in} cRNA (20 ng lane 1), and 20 ng RD-2_o cRNA (lane 2) hybridized with the bridge oligonucleotide probe (5'-GCAGCATCCTTGAGTGGTGTCTTC-3'). The blot was exposed to film for 8 h. *d*, Poly(A)⁺ rat caudate nucleus RNA (7 μ g) hybridized with the insert probe. The autoradiograph was exposed for 5 days. *e*, Poly(A)⁺ rat caudate nucleus RNA (7 μ g) hybridized with the bridge probe. The autoradiograph was exposed for 5 days. *f*, Poly(A)⁺ (rat caudate nucleus RNA (7 μ g) hybridized with an oligonucleotide probe to transmembrane domain V (5'-GGCAGGGTGGCAATGATACACTC-3'). Exposure was for 5 days. *g*, Nucleotide sequences of RD-2_o and RD-2_{in} flanking and including the insert starting with nucleotide 690 (ref. 2). The bridge probe is complementary to the overlined sequence of RD-2_o. The insert probe is complementary to the underlined sequence of RD-2_{in}.

METHODS. RNA was prepared from rat caudate nuclei freshly dissected and quickly frozen in liquid nitrogen (see ref. 17). The poly(A)⁺ RNA was twice purified by oligo(dT) cellulose chromatography¹⁸. RD-2_o and RD-2_{in} cRNAs were synthesized after linearization of the RD-2_o and RD-2_{in} plasmid DNA with *Not*I. Transcription reactions were carried out using T7 RNA polymerase. RD-2_o cDNA was random-primer labelled to a specific activity of $1\text{--}2 \times 10^9$ c.p.m. per μ g. The oligonucleotide probes were end-labelled with T4 polynucleotide kinase¹⁹ to specific activities of $5\text{--}9 \times 10^8$ c.p.m. μ g⁻¹. Hybridization was according to ref. 20 using 1×10^7 c.p.m. ml⁻¹ at 65 °C in 0.25 M Na₂HPO₄, 1 mM EDTA, 1% BSA, 7% SDS.



Northern blots incubated with oligonucleotide probes were washed with 20 mM Na₂HPO₄, pH 7.2, 1 mM EDTA, 0.5% BSA, 5% SDS at 58 °C and 20 mM Na₂HPO₄, 1 mM EDTA, 1% SDS at 50 °C. The northern blot incubated with random-primer-labelled probe was washed in the buffers described above at 65 °C.

distinguish between D-2_o and D-2_{in} messenger RNA. To assess the relative abundance of the two types of RNA transcripts, we designed oligonucleotide probes that selectively hybridize to either D-2_o or D-2_{in} mRNA. The D-2_{in}-specific probe is specific for the insert region, and the D-2_o-specific probe bridges the sequences flanking the insert region (Fig. 4g). We established conditions that yielded selective hybridization of each type of probe to the appropriate type of transcript using northern blots of complementary RNA synthesized from the RD-2_o and RD-2_{in} clones (Fig. 4b, c). Hybridization of the insert probe with rat caudate nucleus RNA gave a band corresponding to 3.2 kb (Fig. 4d), the same size as the transcript identified by random-primer-labelled D-2_o DNA (Fig. 4a). Hybridization of the bridge probe with the same amount of rat caudate nucleus RNA yielded a much weaker signal (sevenfold less by densitometry), also at a position corresponding to 3.2 kb. An oligonucleotide probe to transmembrane domain V that does not distinguish between either type of RNA, also recognized the 3.2-kb transcript of rat caudate nucleus RNA (Fig. 4f). Frontal cortex and whole-brain mRNA samples showed a faint hybridization signal only with the insert and common probes (data not shown). Similar analysis of RNA prepared from the pituitary and substantia nigra was not possible because of the lower levels of either type of D₂-specific transcripts in these tissues². So at least in the caudate nucleus of the rat, which is the richest and largest brain source of D₂ dopamine receptors⁴, RNA encoding D-2_{in} is much more abundant than RNA encoding D-2_o.

With the differences between the proteins encoded by D-2_o and D-2_{in} in the third intracytoplasmic loop, there is the possibility that the two molecular forms of D₂ receptors interact with different signal transduction pathways mediated by G proteins. Intracellular signalling mediated by D₂ receptors includes inhibition of adenylyl cyclase activity⁷, reduction in Ca²⁺ mobilization stimulated by hormones⁸ and modulation of the activation of K⁺-channels⁹. These signalling events can be mediated by more than one type of G_i or by G_o. Indeed, both G_o and at least one of the G_i proteins co-purify with D₂ dopamine receptors¹⁰.

That both D-2_o and D-2_{in} clones from rat brain are identical in coding and noncoding regions except for the 87 nucleotide insert, indicates that these two forms of the receptor arise by differential splicing of the same gene. A part of the D₂ receptor gene sequenced so far does not extend to the insert region but does have an intron in the coding sequence located 3' to the area of the insert². It is conceivable that there are additional introns in the gene, perhaps two that flank an exon encoding the insert sequence. The presence of introns in the coding region of receptors linked to G proteins is unusual⁵. The possibility of differential splicing to produce two forms of the D₂ dopamine receptor is an intriguing novelty among this family of receptors and allows for other ways of regulating expression of specific receptor subtypes in various tissues.

Note added in proof: A human clone homologous to RD-2_{in} and BD-2_{in} has been recently described²¹. □

18. Aviv, H. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1408-1412 (1972).
19. Cobianni, F. & Wilson S. H. *Meth. Enzym.* **152**, 94-110 (1987).
20. Church, G. M. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **81**, 1991-1995 (1984).
21. Selbie, L. A., Hayes, G. & Shine, J. *DNA* **8**, 683-689 (1989).

ACKNOWLEDGEMENTS. We thank Dr N. Y. Theriault for oligonucleotide synthesis and D. K. Piper for manuscript preparation.

Axonal regeneration in the rat spinal cord produced by an antibody against myelin-associated neurite growth inhibitors

Lisa Schnell & Martin E. Schwab*

Institute for Brain Research, University of Zurich, August-Forel-Strasse 1, CH-8029 Zurich, Switzerland

* To whom correspondence should be addressed

AFTER lesions in the differentiated central nervous system (CNS) of higher vertebrates, interrupted fibre tracts do not regrow and elongate by more than an initial sprout of ~1 mm (refs 1-3). Transplantations of pieces of peripheral nerves into various parts of the CNS demonstrate the widespread capability of CNS neurons to regenerate lesioned axons over long distances in a peripheral nerve environment (for example, see refs 2 and 3). CNS white matter⁴⁻⁶, cultured oligodendrocytes (the myelin-producing cells of the CNS), and CNS myelin itself, are strong inhibitors of neuron growth in culture⁷, a property associated with defined myelin membrane proteins of relative molecular mass (*M_r*) 35,000 (NI-35) and 250,000 (NI-250)⁸. We have now intracerebrally applied the monoclonal antibody IN-1, which neutralizes the inhibitory effect of both these proteins⁹, to young rats by implanting antibody-producing tumours. In 2-6-week-old rats we made complete transections of the cortico-spinal tract, a major fibre tract of the spinal cord, the axons of which originate in the motor and sensory neocortex^{10,11}. Previous studies have shown a complete absence of cortico-spinal tract regeneration after the first postnatal week in rats¹², and in adult hamsters and cats^{13,14}. In IN-1-treated rats, massive sprouting occurred at the lesion site, and fine axons and fascicles could be observed up to 7-11 mm caudal to the lesion within 2-3 weeks. In control rats, a similar sprouting reaction

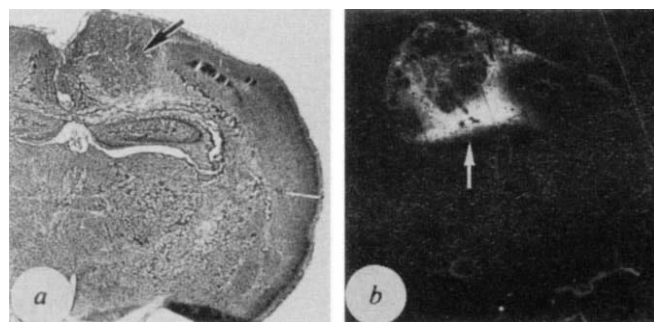


FIG. 1 Tumour of antibody-secreting mouse IN-1-producing hybridoma cells in the cortex of an 8-day-old rat. Cells (10^6) were injected at postnatal (p.n.) day 2. *a*, Cresyl violet-stained frozen section (15 μ m) shows well-circumscribed compact tumour (arrow). *b*, Antibody production demonstrated by immunofluorescence with anti-mouse-FITC-conjugated immunoglobulin. Tumour and surrounding tissue up to the lateral ventricle (small arrow) are strongly stained (adjacent section to that of *a*). Rat was fixed by perfusion with 4% formaldehyde in phosphate buffer. Magnification, $\times 6.4$.

Received 8 September; accepted 22 November 1989.

1. Lee, T., Seeman, P., Tourtellote, W. W., Farley, I. J. & Hornykeiwicz, O. *Nature* **274**, 897-900 (1978).
2. Bunzow, J. R. et al. *Nature* **336**, 783-787 (1988).
3. Strader, C. D. et al. *J. biol. Chem.* **262**, 16439-16443 (1987).
4. Boyson, S. F., McGonicle, P. & Molinoff, P. B. *J. Neurosci.* **6**, 3177-3188 (1986).
5. O'Dowd, B. F., Lefkowitz, R. J. & Caron, M. G. A. *Rev. Neurosci.* **12**, 67-83 (1989).
6. Huff, R. M. & Molinoff, P. B. *J. Pharmac. exp. Ther.* **232**, 57-62 (1985).
7. DeCamilli, P., Macconi, D. & Spada, A. *Nature* **278**, 252-254 (1979).
8. Schofield, J. G. *FEBS Lett.* **159**, 79-82 (1983).
9. Saith, S., Bicknell, R. J. & Schofield, J. G. *FEBS Lett.* **148**, 27-30 (1982).
10. Elazar, Z., Siegel, G. & Fuchs, S. *EMBO J.* **8**, 2352-2357 (1989).
11. Fainberg, A. P. & Vogelstein, B. *Analyt. Biochem.* **132**, 6-13 (1983).
12. Chomczynski, P. & Qasba, P. K. *Biochem. biophys. Res. Commun.* **122**, 340-344 (1984).
13. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463-5467 (1977).
14. Scatchard, G. *Ann. N.Y. Acad. Sci.* **51**, 660-671 (1949).
15. McCutchan, J. H. & Pagano, J. S. *J. natn. Cancer Inst.* **41**, 351-357 (1968).
16. Sompayrac, L. M. & Danna, K. J. *Proc. natn. Acad. Sci. U.S.A.* **78**, 7575-7578 (1981).
17. Chomczynski, P. & Sacchi, N. *Analyt. Biochem.* **162**, 156-159 (1987).

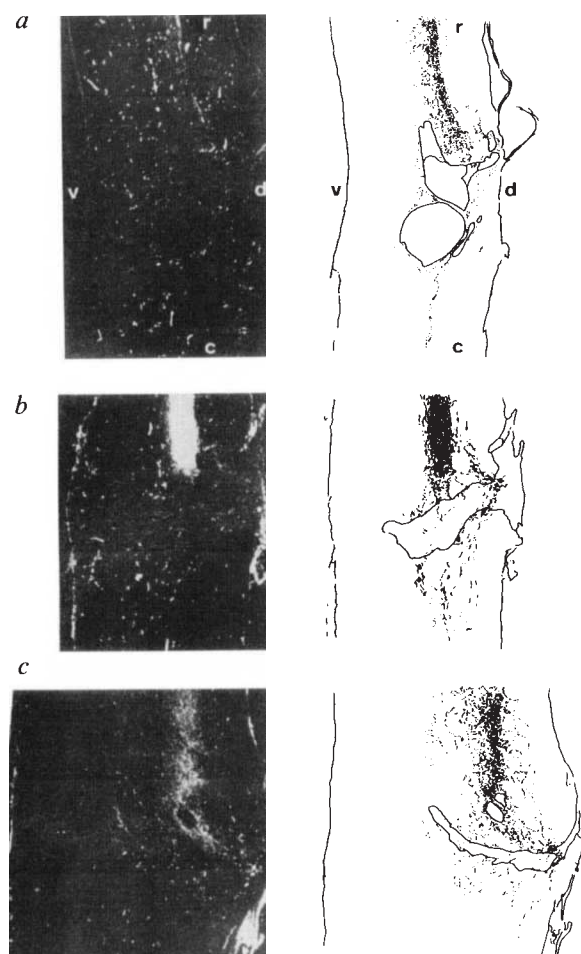


FIG. 2 Examples of lesion sites (longitudinal spinal cord sections) with labelled CST axons; dark-field micrographs and corresponding camera-lucida drawings. Drawings include 3–7 adjacent sections. All points on drawings and the fine points on micrographs show label in CST fibres. Large dots on micrographs represent erythrocytes (catalase) in blood vessels or on spinal cord surface. Note the CST sprouting rostral to the lesion, and regenerating CST fibres passing ventrally (a) or laterally (a–c) to the lesion caverns. A dorsal tissue bridge with CST axons is present in a. a, IN-1-treated rat, lesioned at p.n. day 15; survival time, 14 days. b, IN-1-treated rat, lesioned at p.n. day 47; survival time, 18 days. c, Anti-HRP-treated rat, lesioned at p.n. day 47; survival time, 18 days. Abbreviations r, rostral; c, caudal; d, dorsal; v, ventral. Magnification, $\times 45$.

METHODS. Lewis rats were injected unilaterally under ether anaesthesia into the dorsal frontal cortex with 1×10^6 hybridoma cells in 1 or 2 μ l Hank's solution. Hybridoma cells: IN-1-secreting cells as described previously⁹; anti-HRP-secreting cells were obtained by Dr P. Streit (Zurich) according to the protocol of ref. 19, using the same myeloma line (P3U) as for IN-1. These antibodies were originally produced for peroxidase-anti-peroxidase (PAP) complexes, and do not inhibit HRP enzymatic activity. Anti-HRP-producing tumours were often larger than the IN-1-producing tumours. Antibody penetration in the tissue should be better for anti-HRP (IgG) than for IN-1 (IgM) antibodies. Cyclosporin A (15 mg kg^{-1}) was given intraperitoneally 3 and 6 days after the cell injections. Spinal cord lesions were made at 2–7 weeks of age (Table 1) at the thoracic level T₅₋₇ by slightly separating two vertebrae and transecting the dorsal two-thirds of the spinal cord with iridectomy scissors. A 'U'-shaped stainless-steel wire was then implanted into the lesion site to assure complete transection of both CSTs and to mark the lesion site²⁰. (The wire was removed before embedding the fixed spinal cords for sectioning.) After survival times of 14–33 days (Table 1), the frontal and parietal cortex contralateral to the tumour was injected with a 5% solution of wheat germ agglutinin-HRP (1μ l). Rats were perfused 24 h later through the heart, with 1.25% glutaraldehyde and 1% formaldehyde in 0.1 M phosphate buffer for 10 min. The dissected spinal cords (10–20 mm) were postfixed in the same fixative for 1 h, extensively washed, and embedded for cryostat sectioning. Complete longitudinal section series were mounted on gelatin-coated slides, and reacted for HRP using tetramethyl-benzidine as a substrate²¹. Number-coded section series were viewed under dark-field illumination in polarized light. All rats with incomplete lesions were discarded. Only rats with sprouts appearing on the caudal side of the lesion were evaluated.

TABLE 1 Regeneration of CST axons after spinal cord lesions

Antibody	Day of lesion (postnatal)	Survival time (days)	Maximal distance of regenerated CST axons (mm)	Antibody	Day of lesion (postnatal)	Survival time (days)	Maximal distance of regenerated CST axons (mm)
None	14	19	0.1	IN-1	14	16	2.0
			0.2				>8.0
			0.2				11.0
			0.5	IN-1	15	15	4.0
None	22	14	0.4				4.5
			0.2				>5.0
None	22	11	0.7				>5.0
			0.6				>5.0
anti-HRP	15	14	0.4	IN-1	15	33	2.0
			1.0	IN-1	18	18	2.5
			1.8				2.8
			2.6				>3.0
anti-HRP	18	16	0.1				4.9
			0.2	IN-1	19	14	7.7
			0.2				7.8
			0.3	IN-1	28	14	>4.0
			0.4				>4.0
			0.5	IN-1	29	27	>2.5
			0.8	IN-1	37	14	7.5
	37	14	0.4	IN-1	47	18	>2.0
	47	18	0.8				

Methods were as described in legends to Figs 2 and 4. Evaluations were made blind on number-coded section series. Only rats with regenerative CST sprouts caudal to the lesion were included in this analysis. Distances of regenerating fibres are measured from the caudal edge of the lesion caverns. The symbol > indicates a minimal distance, because regenerating fibres reached the caudal end of the tissue block.

occurred, but the maximal distance of elongation rarely exceeded 1 mm. These results demonstrate the capacity for CNS axons to regenerate and elongate within differentiated CNS tissue after the neutralization of myelin-associated neurite growth inhibitors.

Monoclonal antibodies IN-1 were obtained by immunizing against the PAGE-purified M_r 250,000 inhibitory protein fraction from rat spinal cord myelin as previously described⁹. IN-1-producing hybridoma cells (10^6) were injected unilaterally into the dorsal fronto-parietal cortex of rats 7–10 days before the spinal cord lesion. Control rats were injected with the same number of cells of a hybridoma line producing antibodies against horseradish peroxidase (HRP). Noninjected controls were also used. In all hybridoma-injected rats, solid well-delineated tumours formed within a few days, often spanning the entire thickness of the neocortex and contacting the lateral ventricle (Fig. 1). Injections of cyclosporin A helped to prevent tumour resorption, which otherwise occurred after 1–2 weeks. Production of antibodies was detected by staining brain sections with anti-mouse fluorescein isothiocyanate (FITC)-conjugated immunoglobulin (Fig. 1b), and by the presence of IN-1 antibodies in the serum (data not shown). A continuous supply of antibodies from the cerebro-spinal fluid could therefore be achieved. Lesions were placed at mid-thoracic levels of the spinal cord (Fig. 2 legend) and completely transected the cortico-spinal tracts (CSTs) of both sides, including the lateral projections into the dorsal gray matter and the few CST fibres in the dorso-lateral funiculus¹⁰, and also the central canal. Ventral and ventro-lateral white matter remained largely undisturbed, allowing the rats a seemingly normal behaviour. Lesions were made at 14–47 days of age, that is, 5–37 days after the termination of axon growth in the CST^{11,15}. Two to three weeks after the lesion, the CST was labelled unilaterally by anterograde transport of wheat germ agglutinin-HRP and examined on complete series of longitudinal sections. Section series were number-coded so that there was no possibility of distinguishing IN-1-treated material from control material. All animals with incomplete lesions of the CST were excluded from the analysis.

The lesion sites showed an identical picture in control, anti-HRP-, and IN-1-treated animals: the lesions transected the dorsal half to two-thirds of the spinal cord, and one or several caverns were present, which communicated with the central canal and the subarachnoid space (Fig. 2). The latter feature probably greatly enhanced the local access and penetration of the antibodies from the cerebro-spinal fluid. Labelled CST fibres approached the lesion as a compact bundle from which massive sprouting occurred 0.5–1 mm rostral to the lesion (Fig. 2). In most control or IN-1-injected animals, fibre plexus and fine bundles were seen in and across the lesion area, most often circumventing the lesion caverns ventrally or laterally (Fig. 2). Rarely, fibres grew through tissue bridges that had formed in the wire tract.

Labelled fibres leaving the lesion site and travelling in a caudal direction were frequently observed in control and IN-1-treated rats. But in animals without tumours, and in rats with anti-HRP-producing tumours, these fibres rarely grew more than 1 mm from the distal edge of the lesion site, and no labelled CST fibres were seen beyond 1–2.6 mm caudal to the lesion (Table 1; Fig. 3). This result also strongly confirms the completeness of the CST lesions that were made. By contrast, rats with IN-1-secreting tumours consistently showed labelled fibres or fine fascicles at much farther distances caudal to the lesion (Figs 3 and 4). Labelled CST fibres were found 2.5–5 mm from the lesion site in many animals, and as much as 7–11 mm away in several rats (Table 1). Anatomically, these CST fibres were often close to or in the original CST location, with some fibres also in the gray matter and a few fibres in more dorsal regions. They often followed an irregular course; branching was seen quite frequently (Fig. 4c–i). In most cases they could be traced back to the lesion site on serial sections. The number of these caudal axons was always small, at best a few per cent of the CST axons.

This low number also made it impossible to use markers such as Di-I, which label only a small percentage of the CST neurons.

Two findings strongly indicate that the dorsal CSTs were completely lesioned, and that these fibres caudal to the lesion were truly regenerating CST axons. First, although CST fibres sprouting rostral to and around the lesion occurred in an identical way in tumourless, anti-HRP-, and IN-1-treated rats, CST fibres were mostly confined to the first 1 mm caudal to the lesion in the two groups of controls. Second, the morphology of the CST fibres caudal to the lesion in IN-1-treated animals was peculiar and made them easy to distinguish from normal CST fibres, or from the very few ventral, uncrossed CST fibres. Regenerating fibres grew in an irregular way with frequent branching, and often ended before the end of the tissue block. By contrast, unlesioned fibres were straight and could easily be followed through the whole extent of the tissue block.

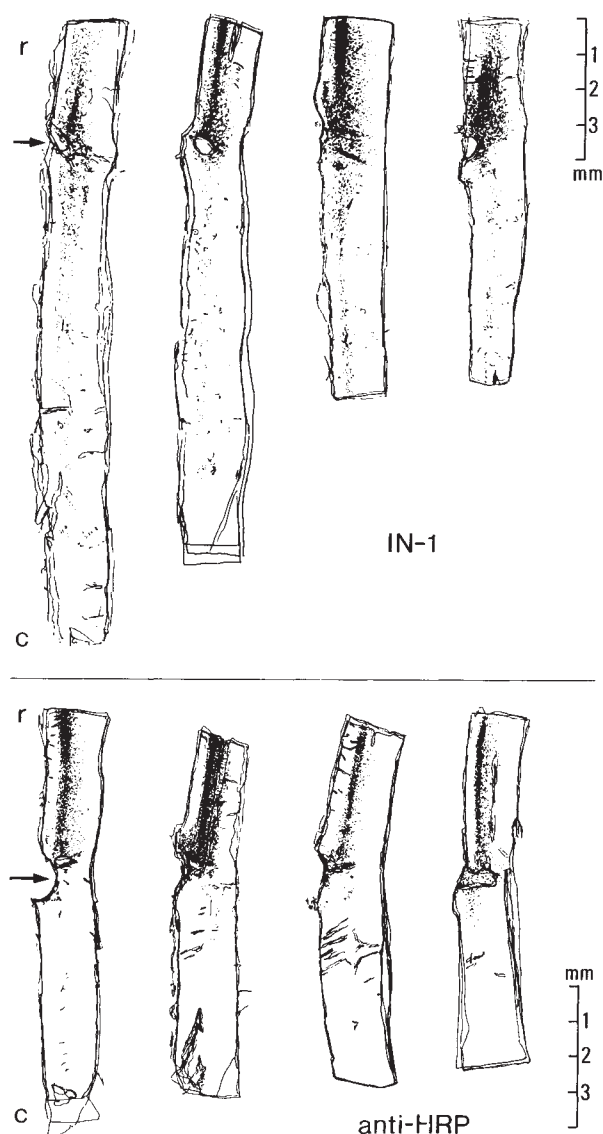


FIG. 3 Labelled cortico-spinal tract fibres are present far-caudal to spinal cord lesions in four IN-1-treated rats (top), but not in four control anti-HRP-treated animals (bottom). Camera-lucida drawings of longitudinal sections of spinal cords (75- μ m total thickness, three superimposed 25- μ m sections) showing wheat germ agglutinin-HRP labelling pattern. Arrows point to lesion sites, which sometimes contain caverns communicating with the central canal. Abbreviations: r, rostral; c, caudal.

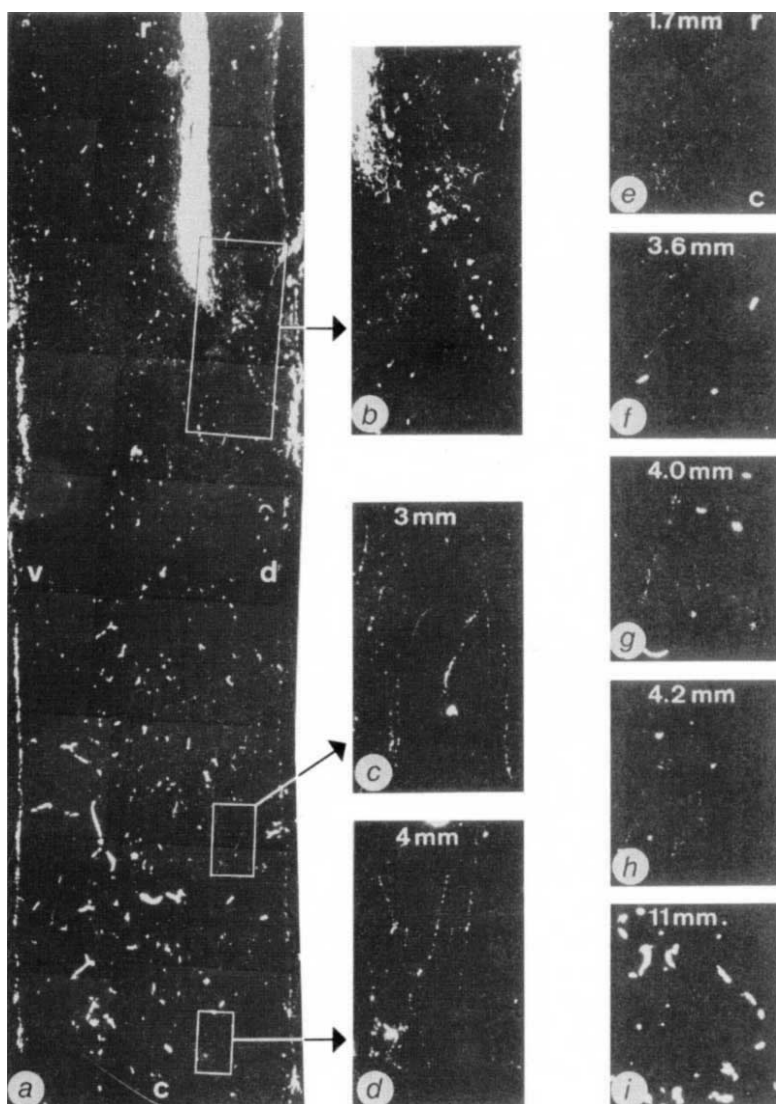


FIG. 4 *a-d*, Montage (*a*) and higher magnifications (*b-d*) of dark-field micrographs of a spinal cord longitudinal section of IN-1-treated rat showing rostrally the unlesioned CST, the sprouting zone rostral to the lesion, and fine-grain-labelled regenerating CST axons caudal to the lesion. Large white dots represent erythrocyte reaction product in blood vessels and on spinal cord surface. Rat was lesioned at postnatal day 37; survival time, 14 days. *e-i*, Regenerating CST axons at given distances caudal to the lesions in different IN-1-treated rats. Note the often irregular course of the fibres. Abbreviations: r, rostral; c, caudal; d, dorsal; v, ventral. Distances from caudal edge of the lesions are given. Magnifications: *a*, $\times 42$; *b*, $\times 90$; *c*, $\times 190$; *d*, $\times 220$; *e-i*, $\times 160$.

It is of interest that the age of the rats in the range chosen (14–47 days) was not critical; even 6–7-week-old rats, that is, fully adult rats, showed long-distance regeneration of CST fibres. By using the longest distances of regeneration observed, a maximal speed of elongation of 0.5–1 mm per day can be estimated for both young and older animals.

Differentiated CNS tissue of mammals, in particular white matter, is a nonpermissive substrate for neurite growth of more than the sprouting distance of 0.2–1 mm^{1–6}. The transplantations of fetal adrenergic or serotonergic neurons into adult spinal cords or hippocampi were, until now, the only experiments in which elongation of axons in adult CNS tissue had been observed on the anatomical level^{16–18}. These axons were almost exclusively localized in areas of gray matter. Two oligodendrocyte- and myelin-associated membrane proteins, NI-35 and NI-250, with potent inhibitory effects on neurite growth, were identified by *in vitro* and biochemical studies^{7,8}. The neutralizing antibody IN-1 (ref. 9) is shown here to lead to regeneration of cortico-spinal axons in young rats over distances of up to 5–11 mm within 2–3 weeks. The absence of axon elongation despite massive sprouting around the lesion site in the controls confirms the completeness of the lesions and the specificity of the effect observed. Very similar results were recently obtained in rats in which myelin formation had been suppressed by neonatal X-irradiation (T. Savio and M.E.S., manuscript in preparation). The small number of successfully regenerating axons could be due to the large extent of the lesion, partial

effects of the antibodies owing to their limited penetration, intrinsic properties of the CST neurons, or to suboptimal trophic factor or substrate conditions. □

Received 7 August; accepted 23 November 1989.

1. Ramon y Cajal, S. *Degeneration and Regeneration of the Nervous System* (Hafner, New York, 1959).
2. David, S. & Aguayo, A. J. *Science* **214**, 931–933 (1981).
3. Vidal-Sanz, M., Bray, G. M., Villegas-Pérez, M. P., Thanos, S. & Aguayo, A. J. *J. Neurosci.* **7**, 2894–2909 (1987).
4. Schwab, M. E. & Thoenen, H. *J. Neurosci.* **5**, 2415–2423 (1985).
5. Carbonetto, S., Evans, D. & Cochard, P. *J. Neurosci.* **7**, 610–620 (1987).
6. Savio, T. & Schwab, M. E. *J. Neurosci.* **9**, 1126–1133 (1989).
7. Schwab, M. E. & Caroni, P. *J. Neurosci.* **8**, 2381–2393 (1988).
8. Caroni, P. & Schwab, M. E. *J. Cell Biol.* **106**, 1281–1288 (1988).
9. Caroni, P. & Schwab, M. E. *Neuron* **1**, 85–96 (1988).
10. Casale, E., Light, A. R. & Rustioni, A. *J. comp. Neurol.* **278**, 275–286 (1988).
11. Joosten, E. A. J., Gribnau, A. A. M. & Dederen, J. W. C. *Devl Brain Res.* **36**, 121–130 (1987).
12. Bernstein, D. R. & Stelzner, D. J. *J. comp. Neurol.* **221**, 382–400 (1983).
13. Kalil, K. & Reh, T. *J. comp. Neurol.* **211**, 265–275 (1982).
14. Tolbert, D. L. & Der, T. *J. comp. Neurol.* **260**, 299–311 (1987).
15. Schreyer, D. J. & Jones, E. G. *Devl Brain Res.* **38**, 103–119 (1988).
16. Nornes, H., Björklund, A. & Stenevi, U. *Cell Tissue Res.* **230**, 15–35 (1983).
17. Foster, G. A. *et al.*, *Expl Brain Res.* **60**, 427–444 (1985).
18. Björklund, A., Segal, M. & Stenevi, U. *Brain Res.* **170**, 409–426 (1979).
19. Semenenko, F. M., Bramwell, S., Sidebottom, E. & Cuello, A. C. *Histochemistry* **83**, 405–408 (1985).
20. Borgens, R. B., Blight, A. R. & Murphy, D. J. *J. comp. Neurol.* **250**, 157–167 (1986).
21. Mesulam, M.-M. *J. Histochem. Cytochem.* **26**, 106–117 (1978).

ACKNOWLEDGEMENTS. We thank Dr Tiziana Savio for help with the operations, Dr P. Streit for the anti-HRP hybridomas, Drs Christine Bandtlow, Danièle Cadelli and Josef Kapfhammer for critically reading the manuscript, Silvia Kaufmann for secretarial help, and Roland Schoeb and Eva Hochreutener for help with the figures. The work was supported by the Swiss National Science Foundation, the Swiss Multiple Sclerosis Society, the Dr E. Slack-Gyr-Foundation (Zurich), the American Paralysis Foundation, and Regeneron Pharmaceuticals, Inc., New York.

Origin of cells giving rise to mesoderm and endoderm in chick embryo

Claudio D. Stern & David R. Canning*

Department of Human Anatomy, South Parks Road, Oxford OX1 3QX, UK

* Present address: Department of Developmental Genetics and Anatomy, Case Western Reserve University, School of Medicine, Cleveland, Ohio 44106, USA

IN amniotes, all of the tissues of the adult arise from the epiblast, one of the two layers of cells present in the early embryo; the mesoderm and gut endoderm arise from an epiblast-derived structure known as the primitive streak. The monoclonal antibody HNK-1¹⁻³ recognizes the cells of the primitive streak in the chick embryo⁴. Before streak formation, HNK-1 identifies cells that are randomly distributed within the epiblast⁴. We have now used two novel ways to study cell lineage and commitment to show that the epiblast of the early chick embryo contains two distinct populations of cells with different developmental fates at a stage during which 'mesodermal induction' is believed to occur. One cell population, recognized by monoclonal antibody HNK-1, is destined to form mesoderm and endoderm; the rest of the epiblast is unable to give rise to mesoderm if this population of cells is removed.

We designed two simple experiments to investigate whether the HNK-1-positive cells found in the epiblast before primitive streak formation are the precursors of the HNK-1-positive cells of the primitive streak (Fig. 1). In the first experiment, we followed the developmental fate of the HNK-1-positive cells by using a novel approach for cell lineage analysis—the labelling of cells expressing the HNK-1 epitope with the antibody coupled to colloidal gold (Fig. 1a). This method enabled us to identify the cells that were HNK-1-positive at the time of labelling regardless of whether or not they continued to express the HNK-1 epitope during subsequent development. After primitive streak formation, virtually all gold-labelled cells were found in mesodermal and definitive-endodermal tissues (Figs 2 and 3). Control embryos labelled with 15-nm colloidal gold, diluted so

that random patches of epiblast cells become labelled, showed no differential distribution of labelled cells among the various tissues after embryo culture (Figs 2 and 3). This experiment shows that the HNK-1-positive cells found in the epiblast of the pre-primitive streak (stage XIII; ref. 5) embryo contribute almost exclusively to tissues derived from the primitive streak.

In the second experiment (Fig. 1b), we ablated the HNK-1-positive cells of the epiblast by using HNK-1 and complement. When embryos treated in this way were placed in a solution of a dye that is only taken by dead cells, a similar mosaic pattern as that seen by HNK-1 immunocytochemistry was revealed in the epiblast (Fig. 4d, e). When grown in culture⁶, control embryos treated with antibody alone or complement alone developed normally (Fig. 4a, b), whereas embryos treated with HNK-1 and complement increased in size normally, but had no primitive streak or mesodermal structures (Fig. 4c). But treated embryos could be made to develop normally if they received a graft of a piece of primitive streak from an untreated chick or quail embryo—that is, a single primitive streak formed (Fig. 4f), and the mesodermal and endodermal cells were derived from the quail graft (Fig. 4g). By contrast, treated embryos grafted with a treated quail epiblast or an untreated hypoblast behaved as if they had not been grafted: they failed to form mesodermal structures. This experiment shows that the epiblast cannot form mesodermal structures when the HNK-1-positive cells are removed unless (HNK-1-positive) primitive streak cells are supplied. It further indicates that after removal of these cells, HNK-1-negative cells in the epiblast are not capable of altering their phenotype to become primitive streak cells.

The epiblast of the chick embryo therefore contains a randomly distributed subpopulation of HNK-1-positive cells at a stage (XIII; ref. 5) preceding formation of the primitive streak; these cells give rise to tissues derived from the primitive streak (mesoderm and endoderm).

C. H. Waddington⁷ showed that antero-posterior rotation of the hypoblast before primitive streak formation reverses the anterior-posterior axis of the streak. Because the hypoblast coalesces to form a sheet of cells in the posterior-anterior direction, it has been inferred from Waddington's results that

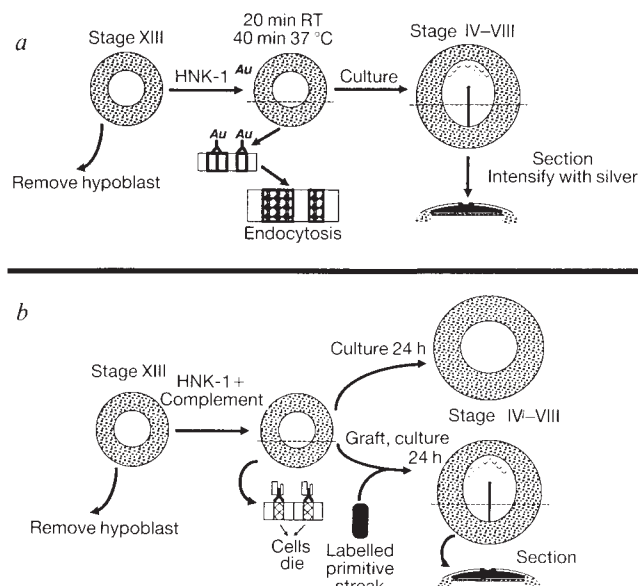


FIG. 1 a, Experimental design for lineage mapping of HNK-1-positive cells. The HNK-1-positive cells of the epiblast were marked using the antibody coupled to colloidal gold, and the embryos allowed to develop in culture until after primitive streak formation. Histology then revealed the position of the original HNK-1-positive cells regardless of whether or not they continued to express the epitope. b, Ablation of HNK-1-positive cells. In this experiment, the HNK-1-positive cells of the epiblast were killed using the antibody and complement. Some of the treated embryos were grafted with HNK-1-positive cells from a quail donor before further development in culture. Histology then revealed the fate of the transplanted quail cells.

METHODS. a Stage XIII (ref. 5) chick embryos deprived of their hypoblast were incubated in HNK-1 covalently coupled to 15 nm colloidal gold (HNK-1^{Au}) for 20 min at room temperature followed by 40 min at 37 °C. During this period the labelled cells endocytose the HNK-1^{Au} complex. They were then washed in Pannett-Compton saline and grown in whole embryo culture to stages 4-8 (ref. 16), formalin-fixed and wax-sectioned and the sections intensified with silver reagent (IntenSE, Janssen). The sections were examined by bright-field microscopy or by confocal laser scanning microscopy in reflection mode. Method for gold-coupling to HNK-1 modified from ref. 17; the optimal pH for adsorption was 8.2. Control embryos treated identically were labelled with 15-nm AuroBeads (Janssen, diluted 1:1 in saline) and then cultured. b, Stage XIII (ref. 5) chick embryos deprived of their hypoblast were incubated in HNK-1 supernatant and guinea pig complement (1:1) for 1 h at 37 °C, washed in Pannett-Compton saline, and set up in whole embryo culture⁶. One set of treated and washed embryos received a graft of about one-third of the mesoderm from a stage 3 (ref. 16) primitive streak from a quail embryo after HNK-1-complement treatment and was then cultured. Control experiments: HNK-1 alone (1:1 in saline), complement alone (1:1 in saline). Grafted embryos were fixed in Zenker's fixative and wax-sectioned, and the sections stained with haematoxylin after acid hydrolysis to visualize the quail cells^{18,19}. RT, room temperature.

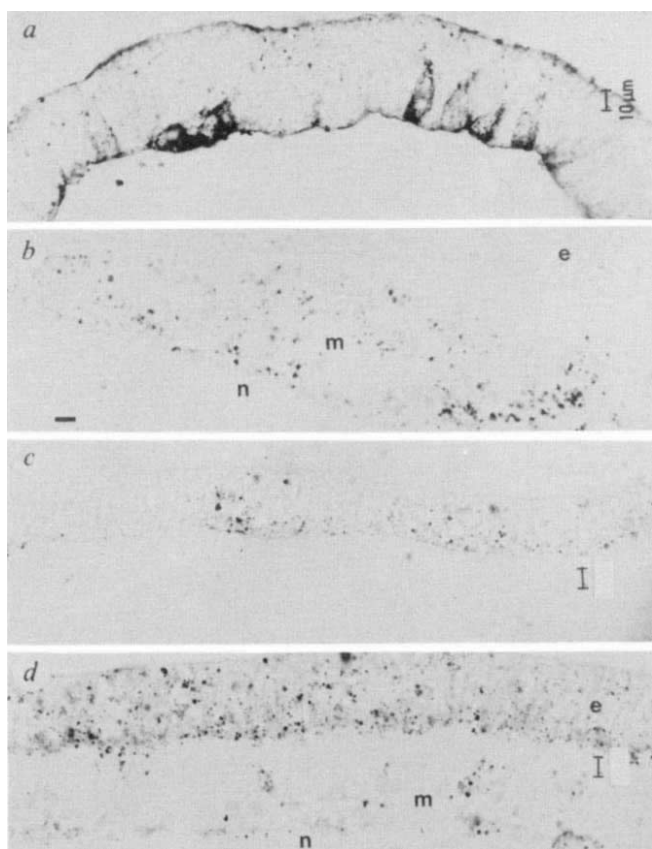


FIG. 2 HNK-1-Au experiment. If embryos were fixed and sectioned immediately after labelling with HNK-1^{Au}, some cells of the epiblast were found to have endocytosed the HNK-1^{Au} complex (a). After further incubation, cells labelled with HNK-1^{Au} ($n=3$ (number 31) embryos) were found in the definitive endoderm and mesodermal tissues but not in the ectoderm (b); scale bar, 10 μ m. Control embryos labelled directly with 15-nm gold particles (c; diluted so that only some epiblast cells became labelled) displayed labelled cells in all tissues after a similar period in whole embryo culture (d). Abbreviations e, ectoderm; m, mesoderm; n, endoderm.

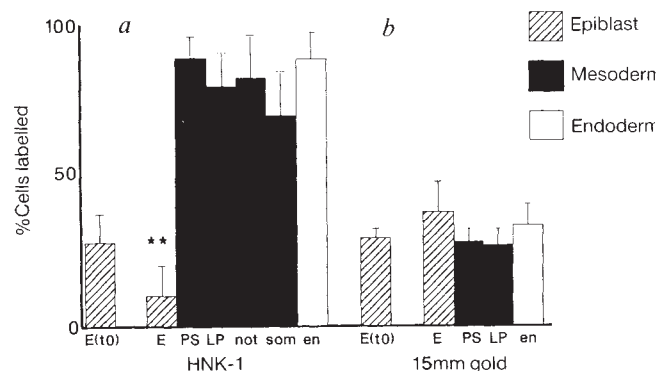


FIG. 3 Quantification of HNK-1-Au experiment. Cells labelled with HNK-1^{Au} were found in mesodermal and endodermal tissues but not in ectoderm. By contrast, cells labelled directly with 15-nm gold were equally distributed among all tissues. The abscissa represents the proportion (%) of labelled cells within a given tissue; counts were obtained from 19 HNK-1^{Au} labelled embryos and five 15-nm-gold-labelled embryos. All of the cells of at least five nonadjacent sections were counted in each embryo. The error bars are standard deviations. E(t0), epiblast immediately after labelling. After culture: E, lateral epiblast; PS, primitive streak (all layers); LP, lateral plate mesoderm; not, notochord; som, somite; en, definitive (gut) endoderm; **, significantly different ($P<0.001$) from each of the other HNK-1^{Au}-labelled tissues and from epiblast labelled with 15-nm gold.

the overlying epiblast is made up of uncommitted cells, and that the hypoblast restricts the fates of the cells with which it first comes into contact at the posterior end of the embryo, so that cells induced by the hypoblast form the primitive streak, whereas uninduced cells remain in the ectoderm and become the precursors of the skin and nervous system⁸⁻¹². The result of Waddington's hypoblast-rotation experiments should be reinterpreted in the light of our findings. Because the HNK-1-positive cells of the epiblast make their appearance in a random distribution among the cells of the epiblast⁴, it seems unlikely that the hypoblast induces mesoderm and endoderm from uncommitted epiblast cells with which it first comes into contact at the posterior end of the embryo.

Two populations of cells contribute to the formation of the hypoblast. At first, small islands of 'primary' hypoblast cells are present in the embryo (stage X; ref. 5); these islands are distributed randomly against the ventral surface of the epiblast. Later (stages XI-XIII; ref. 5), these islands receive a contribution from cells ('secondary' hypoblast) derived from the posterior margin of the embryonic disc. These marginal zone-derived secondary hypoblast cells constitute the tissue that has been postulated to induce the primitive streak^{9,13-15}.

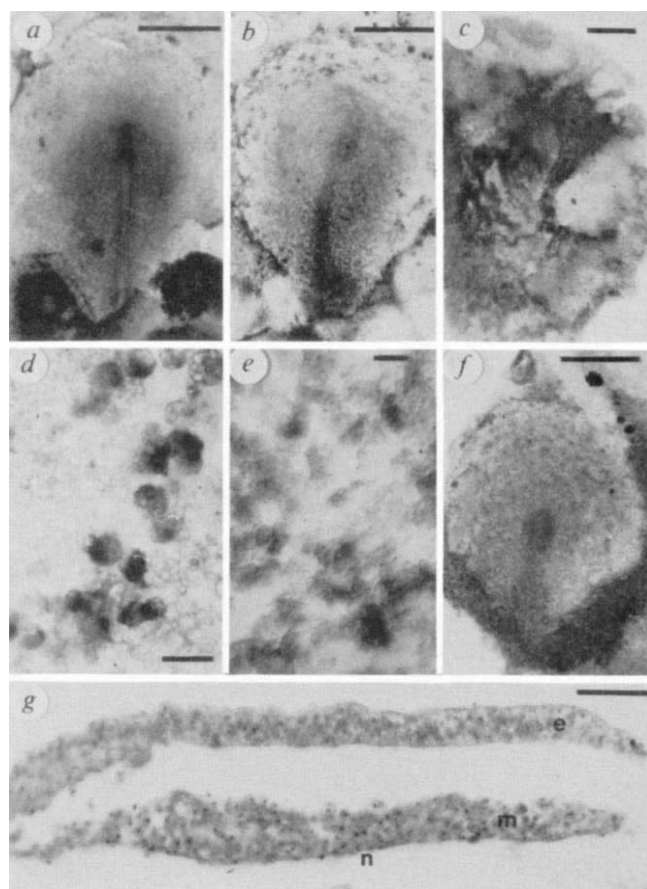


FIG. 4 HNK-1-complement experiment. Embryos treated with HNK-1 antibody alone ($n=10$; a) or complement alone ($n=14$; b) developed normally, whereas those treated with HNK-1 and complement ($n=32$) failed to form a primitive streak (c). HNK-1-complement treatment kills a subpopulation of epiblast cells that can be visualized by brief immersion of the treated embryo in Nigrosin (d); the resulting pattern resembles the pattern of staining seen with HNK-1 at this stage (e; immunoperoxidase). If a piece of chick ($n=10$) or quail ($n=22$) primitive streak is grafted into embryos treated with HNK-1-complement, a single embryonic axis of normal appearance develops (f), and this is composed of donor quail cells (g). Scale bars, 500 μ m (a-c, f); 10 μ m (d, e); 50 μ m (g). Abbreviations e, epiblast; m, mesoderm; n, endoderm.

It is possible that in the experiments we performed, the islands of primary hypoblast present during early development induced neighbouring epiblast cells to become primitive streak cells in a very local way at a stage earlier than stage XIII (ref. 5). But it is clear from Waddington's experiment that the secondary hypoblast plays some part in determining the position at which the primitive streak will form, because at stage XIII (ref. 5) the rotation of the secondary hypoblast, or rotation of the marginal zone from which it is derived, reverses the orientation of the embryo^{7,13}. So one role of the secondary hypoblast must be to mark the site at which the presumptive primitive streak cells will collect. The hypoblast could also be required for the later differentiation of axial mesoderm cells into notochord and somites. An isolated piece of epiblast from the centre of the embryo cannot form axial structures in the absence of both the marginal zone and the hypoblast, but it can form axial structures if supplied with either of these tissues, even if the hypoblast has been separated into single cells¹³⁻¹⁵.

At a stage when the embryo can respond to rotation of the hypoblast or marginal zone by reversal of position of the embryonic axis, the epiblast is composed of a mixture of cells that seem to be committed to become either ectoderm or primitive streak cells. It is therefore clear that the cells of the primitive

streak are not induced by the hypoblast at the posterior margin of the embryo at this stage of development. □

Received 20 July; accepted 5 December 1989

1. Abo, T. & Balch, C. M. *J. Immun.* **127**, 1024 (1981).
2. Tucker, G. C. *et al. Cell Diff.* **14**, 223 (1984).
3. Rickmann, M., Fawcett, J. W. & Keynes, R. J. *J. Embryol. exp. Morph.* **90**, 437 (1985).
4. Canning, D. R. & Stern, C. D. *Development* **104**, 643 (1988).
5. Eyal-Giladi, H. & Kochav, S. *Dev. Biol.* **49**, 321 (1976).
6. New, D. A. T. *J. Embryol. exp. Morph.* **3**, 326 (1955).
7. Waddington, C. H. *Wilhelm Roux Arch. EntwMech. Org.* **128**, 502 (1933).
8. Eyal-Giladi, H. *Cell Differ.* **14**, 245 (1984).
9. Azar, Y. & Eyal-Giladi, H. *J. Embryol. exp. Morph.* **61**, 133 (1981).
10. Nieuwkoop, P. D., Johnen, A. G. & Albers, B. *The Epigenetic Nature of Early Chordate Development* (Cambridge University Press, 1985).
11. Smith, J. C. *Development* **105**, 665 (1989).
12. Gurdon, J. B. *Development* **99**, 285 (1987).
13. Azar, Y. & Eyal-Giladi, H. *J. Embryol. exp. Morph.* **52**, 79 (1979).
14. Mitrani, E. & Eyal-Giladi, H. *Nature* **289**, 800 (1981).
15. Mitrani, E. & Eyal-Giladi, H. *Differentiation* **26**, 107 (1984).
16. Hamburger, V. & Hamilton, H. L. *J. Morph.* **88**, 49 (1951).
17. Goodman, S. L. *et al. J. Microsc.* **123**, 201 (1981).
18. Hutson, J. M. & Donahoe, P. K. *Stain Technol.* **59**, 105 (1984).
19. Stern, C. D. & Keynes, R. J. *Development* **99**, 261 (1987).

ACKNOWLEDGEMENTS. This study was supported by the Wellcome Trust (C.D.S.). The confocal microscope was purchased with an MRC grant to R. W. Guillery, C. D. Stern and A. M. Tolkovsky. D.R.C. was an MRC student. We are grateful to G. J. Carlson for technical assistance and to Professors John Gurdon and Lewis Wolpert and Drs Mike Bate, Rosa Beddington, Jonathan Cooke, Charles French-Constant, Roger Keynes, Alfonso Martinez-Arias, Eduardo Mitrani, Jonathan Slack, Jim Smith and Aviva Tolkovsky for stimulating discussions.

Identification of classical minor histocompatibility antigen as cell-derived peptide

Hans-Joachim Wallny & Hans-Georg Rammensee*

Max-Planck Institut für Biologie, Abteilung Immunogenetik, Corrensstrasse 42, 7400 Tübingen, FRG

HISTOCOMPATIBILITY antigens expressed on tissue grafted between individuals are recognized by host T cells, which reject the graft^{1,2}. The major histocompatibility complex (MHC) antigens have been identified on the molecular level, whereas the molecules representing the remaining ones, the minor histocompatibility antigens, are unknown, apart from some exceptions³⁻⁵. The cytotoxic T lymphocyte (CTL) response against minor histocompatibility antigens shares many aspects with that against virus-infected cells^{6,7}. Virus-specific CTL recognize peptides derived from viral proteins produced in the infected cell. These peptides are presented by MHC class I molecules, as indicated by functional and crystallographic data^{8,9}. By analogy, minor histocompatibility antigens have been postulated to be peptides derived from normal cellular proteins presented by MHC class I molecules¹⁰⁻¹². Here we report that peptides derived from normal cellular proteins can indeed be recognized by CTL raised in the classical minor histoincompatible mouse strain combination, C57BL/6 against BALB.B. Thus, we have proven the above postulate, and isolated one of the minor histocompatibility molecules elusive for several decades.

Minor histocompatibility-specific CTL lines (which are widely accepted as a contemporary tool for the detection of minor histocompatibility antigens) were raised by repeatedly stimulating spleen cells from immunized C57BL/6 (abbreviated to B6; H-2^b)-strain mice with multiple minor histoincompatible BALB.B (H-2^b) stimulator cells (the mouse MHC is called H-2, corresponding to the human HLA). Specificity of three independently derived lines and one clone is shown in Table 1. Lines B16X9/1a and B30X9/4a contain MHC class I K^b-restricted CTL and are specific for BALB minor histocompatibility anti-

gens, as shown by recognition of recombinant inbred strains and by F₁ complementation. The third line, B21W9/1a, is specific for an H-4^b determinant expressed on BALB.B cells and is also MHC-restricted. The clone mapki 1, derived from the B16X9/1a line, is K^b-restricted. These CTL were screened for recognition of peptides produced by enzymatic digestion with endoproteinase Lys C (which specifically hydrolyzes peptide bonds at the carboxylic site of lysine) of a protein extract derived from BALB/c spleen cells. CTL were tested on B6-derived EL4 target cells incubated with enzymatic fragments, analogous to the assay system used to define CTL epitopes with synthetic viral peptides⁸. CTL line B16X9/1a, but not B21W9/1a, recognized material eluting at a distinct position of reversed-phase HPLC elution profile (Fig. 1a, b). Antigenic material from another protein digest was chromatographed on several dimensions including gel filtration (Fig. 1c), revealing the relative molecular mass (*M_r*) of the antigen to be ~1,600 (Fig. 1d). In a final separation by reversed-phase HPLC, the antigen eluted as a single sharp peak (Fig. 1e, f). An attempt to sequence this material indicated that it consisted of more than one peptide species of at most 15 amino acids.

In subsequent experiments, protein extracts derived from BALB.B, B6, or BALB/c spleen, or from the BALB/c-derived B-cell tumour J558, were digested in the same way. The BALB.B, BALB/c and J558 material recognized by B16X9/1a CTL eluted at the same position (around fraction 33) as in Fig. 1a, b, whereas B6 material at this and adjacent fractions was not recognized (Fig. 2a-d). CTL line B30X9/4a, but not B21W9/1a, recognized the same active fractions (data not shown). A mock-digest of BALB/c splenic proteins without endoproteinase Lys C, and a self-digest of this enzyme, contained no active fractions (Fig. 2e, f). Peak fractions 33 of BALB.B, B6, BALB/c and J558 material were retested in titrated amounts. CTL lines B16X9/1a as well as B30X9/4a recognized BALB.B-derived material in a dose-dependent fashion down to a dilution of <1 in 300 (Fig. 3a). B6-derived material was not recognized by any of the CTL lines (Fig. 3b). Antigenic determinants are of peptidic nature, as proteases of broad specificity, pronase E and proteinase K, destroy these epitopes in BALB/c and J558 material (Fig. 3c). The K^b-restricted CTL clone mapki 1 (derived from B16X9/1a, see Table 1) recognizes material from the sharp peptide peak shown in Fig. 1e in a dose-dependent manner (Fig. 3d). K^b-restricted recognition of minor histocompatibility peptide by

* To whom correspondence should be addressed.

clone mapki 1 is further indicated by the failure of P815 (H-2^d) target cells to present this peptide (Fig. 3e) and by blocking of lysis by K^b-specific but not D^b-specific antibodies (Fig. 3f). Thus, a minor histocompatibility antigen recognized on BALB.B target cells by K^b-restricted B6-derived CTL can be a peptide derived from a cellular protein irrespective of the MHC haplotype of the cell expressing the minor histocompatibility protein.

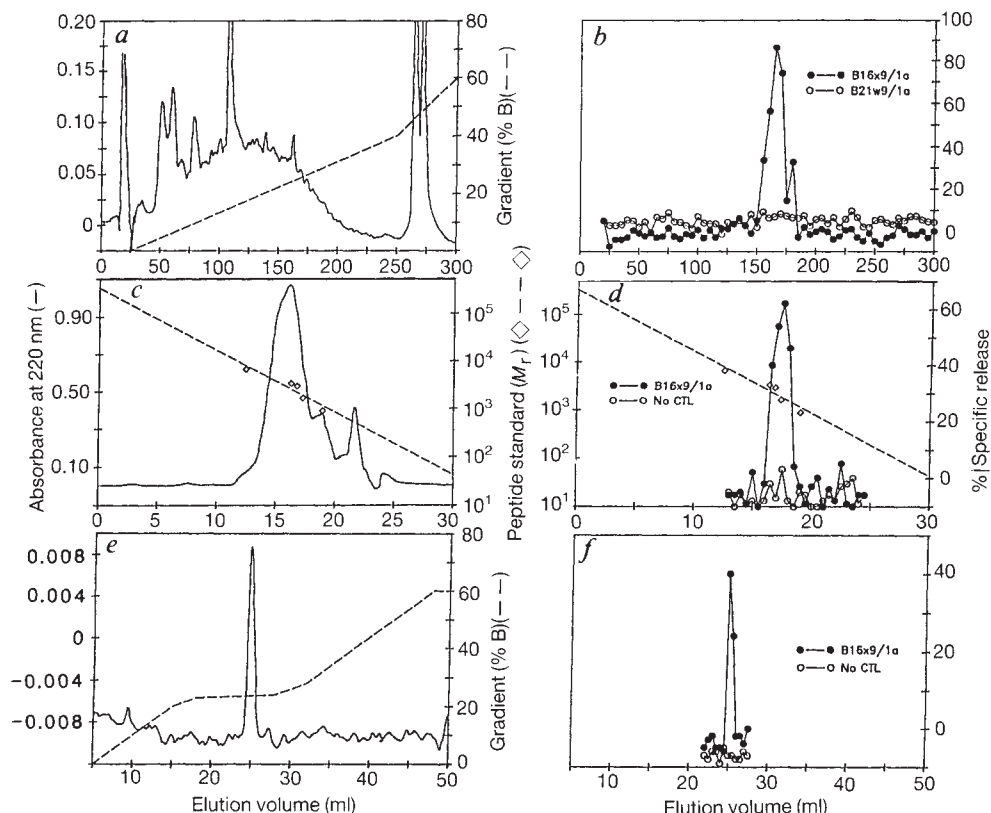
We do not know the chromosomal location of the relevant minor histocompatibility gene. It is not in the H-3 region and, therefore, it is not β_2 -microglobulin, and it is not H-4, as evident from the specificity of the CTL lines used. Most probably, this minor histocompatibility gene product is identical to the most dominant CTL epitope observed in B6 anti-BALB.B CTL (ref. 5).

The identification of those few minor histocompatibility molecules known so far was based on special circumstances in

every case. The first one to be described was β_2 -microglobulin, whose property to reversibly bind to MHC class I heavy chains was a prerequisite for its identification as a minor histocompatibility antigen¹³. Other minor histocompatibility antigens have been identified as retroviral products introduced naturally or artificially into mice (reviewed in refs 4 and 5). The data presented here, however, represent the isolation of a classical minor histocompatibility antigen as a molecule by using an approach not dependent on any special circumstance. We hope to be able to identify the respective minor histocompatibility gene on sequencing the antigenic peptide after further purification. This peptide presumably ends with a lysine residue as judged from the specificity of the protease used to produce it.

A third group of antigens defined by MHC class I-restricted CTL—in addition to viral and minor histocompatibility antigens—are tumour-specific antigens. Several of the latter have been defined by Boon *et al.* as peptides derived from cellular

FIG. 1 EL4 target cells incubated in fractions of enzyme-digested BALB/c proteins are recognized by B16X9/1a CTL. *a*, Saline-soluble proteins extracted from BALB/c spleen cells were enzyme-digested and separated by reversed-phase HPLC. Solid line, elution profile; dashed line, gradient. *b*, ⁵¹Cr-labelled EL4 (B6-derived; H-2^b, MHC class II-negative) tumour target cells were preincubated with individual fractions and tested for recognition by CTL lines B16X9/1a (●) or B21W9/1a (○). *c*, Proteins of J558 tumour cells (BALB/c-derived) were enzyme-digested and separated first by reversed-phase HPLC, then by ion-exchange chromatography. Active fractions were gel-filtrated. Solid line, elution profile; dashed line, peptide standards. *d*, Lysis of EL4 cells incubated with these fractions by B16X9/1a CTL (●) or no CTL (○). *e*, Active fractions of gel filtration were re-chromatographed by reversed-phase HPLC under conditions giving high resolution. Solid line, elution profile; dashed line, gradient. *f*, Fractions were tested as before. Spontaneous ⁵¹Cr-release of target cells ranged from 7.5 to 24.0%. METHODS. Nucleated BALB/c spleen cells (4×10^8) suspended in 2.5 ml PBS were lysed by repeated freezing in liquid nitrogen and thawing ($\times 10$). Supernatant collected after 30-min



centrifugation at 16,500g was dialysed overnight at 4 °C against 50 mM Tris-Cl buffer at pH 8.2, and digested for 7 h at 37 °C with 3 U Lysobacter enzymogenes-derived endoproteinase Lys C (Boehringer), which cuts proteins after lysine residues. Proteinase activity was controlled for by digesting in parallel a sample of spleen proteins together with azocasein (Sigma), followed by spectrographic determination of azocasein fragments, as previously described¹⁹. Enzymatic digestion was stopped by freezing at -70 °C. For separation, the material was chromatographed on a semipreparative reversed-phase column (PepRPC HR 16/10; Pharmacia-LKB) by HPLC using Pharmacia-LKB equipment. Eluting gradient is indicated in Fig. 1a. Solution A, H₂O bidest with 0.1% trifluoroacetic acid (TFA); solution B, acetonitrile with 0.1% TFA. Flow rate, 5 ml/min⁻¹; fraction size, 5 ml. Fractions were dried in a Speed Vac centrifuge (Savant) and resububilized in 0.5 ml PBS by sonication for 15 min in an ultrasound waterbath (Brandelin). For the experiments described in C-F, 4×10^9 J558 tumour cells were treated in essentially the same way, except that trypsin was used for digestion (trypsin cuts protein not only after lysine, but also after arginine residues). Active fractions of this preparation eluting from the semipreparative HPLC column were

subjected to ion-exchange chromatography on a Mono S column (Pharmacia-LKB). Active fractions from this run were separated on an HPLC gel-filtration column (Superose 12, Pharmacia-LKB) using 50 mM Tris-Cl, pH 8.2, as eluent. Flow rate, 0.5 ml min⁻¹; fraction size, 0.5 ml. The column was calibrated with synthetic peptides of *M*_r 880, 1,620, 2,900, 3,300 and 6,600, respectively. Active fractions were further separated by two subsequent reversed-phase HPLC runs on an analytical column (Superpac pepS; Pharmacia-LKB), using the same eluting solutions as used for the semipreparative column, but a different gradient (see e). Flow rate, 1 ml min⁻¹; fraction size, 0.5 ml. Fractions eluting between 20% to 30% of solution B were dried and resububilized in 100 μ l PBS. The chromatogram shown in e is a differential profile calculated with WAVESCAN software (Pharmacia-LKB) by subtracting the profile obtained with running PBS only, from that obtained with the active samples. CTL assays: 10^4 ⁵¹Cr-labelled EL4 target cells were incubated for 90 min at 37 °C with 20 μ l individual fractions in microtitre wells adjusted to 100 μ l with culture medium. CTL were added at an effector:target ratio between 6:1 and 11:1 to give a total microculture volume of 200 μ l. Further protocol, such as for CTL assays, as described in Table 1 legend.

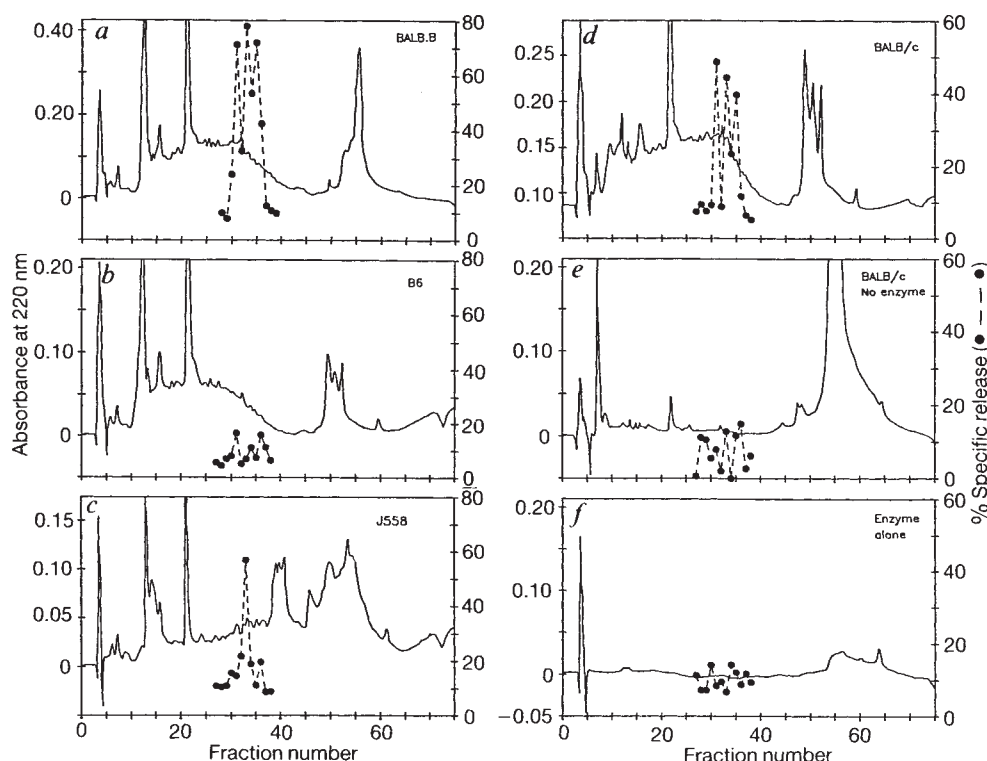


FIG. 2 Isolation of minor histocompatibility antigen from different inbred mouse strains and from a tumour line. *a-d*, Protein fragments produced essentially as described in Fig. 1*a* were incubated with EL4 target cells and tested for recognition by B16X9/1a CTL as in Fig. 1*b*, except that only fractions between nos 27 and 39 were tested (corresponding to 135 ml and 195 ml elution volume, respectively). *a*, Protein fragments derived from six BALB.B spleens. *b*, Fragments from six B6 spleens. *c*, Fragments derived from 3×10^8 J558 (BALB/c-derived tumour cells). *d*, Fragments from six BALB/c spleens. *e*, Proteins from three BALB/c spleens were mock-digested in the absence of endoproteinase Lys C. *f*, Endoproteinase Lys C (1 U) was self-digested in the absence of mouse protein. *e* and *f*, Fractions were resolubilized in 200 μ l PBS. Effector:target ratios were between 5:1 and 7:1. Spontaneous ^{51}Cr -release of target cells ranged between 8.1% and 28.8%.

proteins, which differ from parental protein by amino-acid exchanges due to somatic mutation¹⁴. Thus, it seems that CTL can recognize peptides derived from any aberrant cellular protein, be it due to viral infection, mutations in autologous cells, or to polymorphic proteins in foreign MHC-matched cells (that is, minor histocompatibility antigens). The cellular compartment of such proteins has been thought to be intracellular or even exclusively cytosolic^{11,12,15}. We have shown, however, that pep-

tides derived from proteins expressed in organelles, in the cytosol, or as membrane-inserted or secreted products, can be recognized by a single CTL line¹⁶. Thus, CTL have the potential to survey any protein expressed in a cell for amino-acid exchanges. This implicates that MHC class I molecules, which are expressed on most somatic cells in vertebrates, have the function of presenting peptide samples of cellular proteins to CTL to be tested for aberrance from normal proteins. Viral

TABLE 1 Specificity of B6 anti-BALB.B CTL lines

Target cell				Other minor histocompatibility genes	Lysis by CTL lines*			
	H-2	H-3	H-4		B16X9/1a (imm. BALB.B) [†]	clone mapki 1	B30X9/4a (imm. DBA/2)	B21W9/1a (imm. B10.129-H-4 ^b)
BALB.B	bbbb [‡]	c	§	BALB	78/56/32	31/25/21	62/57/43	55/26/29
C57BL/6	bbbb	a	a	C57BL	1/1/-3	-10/-8/-2	6/13/5	-3/8/-6
BALB/c	dddd	c	§	BALB	-4/7/-3	ND	-9/1/-2	10/11/-3
BALB.5R	bbbd	c	§	BALB	60/36/38	20/24/15	41/41/42	34/23/7
BALB.HTG	dddb	c	§	BALB	ND/39/37	-4/-1/0	-10/8/-10	9/1/-3
DBA/2	dddd	§	§	DBA	2/-9/-10	ND	-10/6/-10	ND
B10.C-H-3 ^c	bbbb	c	a	C57BL	2/-5/3	ND	ND	ND
B10.LP-H-3 ^b	bbbb	b	a	C57BL	-6/8/-4	ND	-12/0/-4	ND
B10.129-H-4 ^b	bbbb	a	b	C57BL	ND	ND	ND	41/44/43
(B10.C-H-3 ^c × BALB/c)F ₁					16/23/18	ND	ND	ND
(B6 × DBA/2)F ₁					ND	ND	30/19/13	ND

CTL lines B16X9/1a, B30X9/4a and B21W9/1a and the clone mapki 1 were tested in a standard ^{51}Cr -release assay on target cells of the mouse strains indicated. Data are pooled from six separate experiments. Starting effector:target cell ratios were between 32:1 and 45:1 for B16X9/1a, between 50:1 and 81:1 for mapki 1, between 19:1 and 35:1 for B30X9/4a, and between 21:1 and 25:1 for B21W9/1a. Spontaneous release of target cells ranged between 11.3% and 32.0%. Information about mouse strains from refs 17 and 18.

B6 mice were immunized intraperitoneally with 2×10^7 spleen cells of BALB.B (for B16X9/1a) or DBA/2 (for B30X9/4a) or B10.129-H-4^b (for B21W9/1a) strain mice. Two-to-four weeks later, 2×10^7 recipient spleen cells were stimulated *in vitro* against 2×10^7 irradiated (3,300 rad) BALB.B spleen cells in Falcon 3013 flasks (Becton-Dickinson) in 10 ml α -minimal essential medium (GIBCO) containing 10% FCS, 2- β -mercaptoethanol, glutamine, and antibiotics in a 5% CO₂ atmosphere at 37 °C. Surviving cells were re-stimulated weekly in medium containing interleukin-2. The clone mapki 1 was derived from B16X9/1a cells by limiting dilution at one cell per well. CTL assays were done according to standard methods³. In brief, concanavalin-A-induced splenic blast cells of the strains indicated were labelled with ^{51}Cr and incubated with titrated numbers of effector cells in 96-well microtitre plates (Greiner) in 0.2 ml culture medium for 4-6 h. Radioactivity released into the supernatant was determined and used as a measure of lysis.

* % Specific lysis at relative effector cell numbers 1, $\frac{1}{3}$, $\frac{1}{9}$.

[†] Strain used to immunize B6 responder mice.

[‡] Alleles at H-2 K, A, E and D/L loci, respectively.

§ Not a or b alleles. H-4^b-specific CTL, however, react with BALB.B (H.G.R., unpublished results).

ND, not determined.

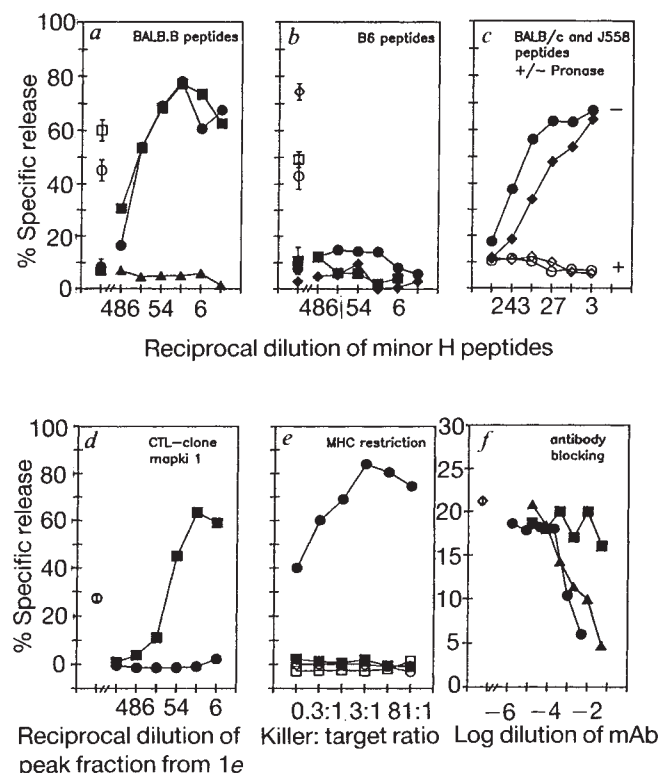


FIG. 3 Characterization of minor histocompatibility peptides. *a, b*, Fractions no. 33 each of BALB.B (*a*) and B6 (*b*) digests (see Fig. 2*a, b*) were incubated in threefold titrations with EL4 target cells (filled symbols) and tested either for recognition by B16X9/1a (●, ○), B30X9/4a (■, □) or B21W9/1a (◆, ◇) CTL, or without CTL (▲). BALB.B target cells were included as a positive control for CTL activity (open symbols). *c*, Fractions no. 33 each of BALB/c (●, ○) and J558 (◆, ◇) digests (see Fig. 2*c, d*) were left untreated (filled symbols) or were pretreated with a mixture of pronase E and proteinase K for 3 h at 37 °C in PBS (open symbols) and tested for recognition on EL4 target cells by B16X9/1a CTL. *d*, Fraction corresponding to 25-ml elution volume in Fig. 1*e* was incubated in threefold titration with EL4 cells (filled symbols) and tested for recognition by clone mapki 1 (see Table 1) (■) or with no CTL (●); BALB.B targets, (○). *e*, EL4 (H-2^b) (●, ○) or P815 (DBA/2-derived tumour, H-2^b) (■, □) target cells were incubated in a 1:6 dilution of fraction no. 35 from Fig. 2*a* (filled symbols) or with medium (open symbols) and tested for recognition by clone mapki 1. *f*, EL4 target cells were preincubated with a suboptimal concentration of BALB.B-derived minor histocompatibility peptide, followed by incubation with antibodies 5F.1 (anti-K^b) (▲), Y-3 (anti-K^b) (●) or H141-51 (anti-D^b) (■) or no antibody (◇), and tested for recognition by clone mapki 1. This experiment was repeated four times with the same result. Significant antibody-blocking at optimal concentrations of minor histocompatibility peptide was not observed. Effector:target ratios in *a-d* and *f* were between 5:1 and 12:1. Spontaneous ⁵¹Cr-release of target cells ranged between 8.1% and 21%.

METHODS. CTL assays as in Fig. 1 and in Table 1. Antibody-blocking: EL4 target cells preincubated with minor histocompatibility peptide were incubated with fivefold dilutions of ascites fluid from the following monoclonal antibodies: 5F.1 (anti-K^b), Y-3 (anti-K^b; ref. 20); H141-51 (anti-D^b; ref. 21). After 30 min of antibody incubation, CTL were added. Abbreviations: H, histocompatibility; mAb, monoclonal antibody.

infection is the best-documented cause for such aberrant proteins; how important CTL are *in vivo* for recognition of tumour-specific antigens that arise by somatic mutation is not known. Minor histocompatibility peptides, as described here, are probably never relevant in nature (the fetal-maternal relationship could be an exception); they are, however, the cause of acute or chronic rejection of grafts in human transplantation, when donor and recipient are matched for MHC determinants. Thus, identification of human minor histocompatibility peptides and genes will be of significance for clinical transplantation. □

Received 25 September; accepted 6 November 1989.

1. Snell, G. D. *Genetics* **49**, 87-108 (1948).
2. Klein, J. *Natural History of the Major Histocompatibility Complex* (Wiley, New York, 1986).
3. Counce, S., Smith, P., Barth, R. & Snell, G. D. *Ann. Surgery* **144**, 198-204 (1956).
4. Loveland, B. & Simpson, E. *Immun. Today* **7**, 223-229 (1986).
5. Wettstein, P. J. in *Human Immunogenetics* (ed. Litwin, S. D.) 339-357 (Dekker, New York, 1989).
6. Zinkernagel, R. M. & Doherty, P. C. *Nature* **248**, 701-702 (1974).
7. Bevan, M. J. *Nature* **256**, 419-421 (1975).
8. Townsend, A. R. M. et al. *Cell* **44**, 959-968 (1986).
9. Bjorkman, P. J. et al. *Nature* **329**, 512-518 (1987).
10. Clavierie, J.-M. & Kourilsky, P. *Ann. Inst. Pasteur, Paris* **137D**, 425-442 (1986).
11. Germain, R. *Nature* **322**, 687-689 (1986).
12. Bevan, M. J. *Nature* **325**, 192-194 (1987).
13. Rammensee, H. G., Robinson, P. J., Crisanti, A. & Bevan, M. J. *Nature* **319**, 502-504 (1986).
14. De Plaen, E. et al. *Proc. natn. Acad. Sci. U.S.A.* **85**, 2274-2278 (1988).
15. Carbone, F. R. & Bevan, M. J. *J. exp. Med.* **169**, 603-612 (1989).
16. Rammensee, H.-G., Schild, H. & Theopold, U. *Immunogenetics* **30**, 296-302 (1989).
17. Klein, J., Figueroa, F. & David, C. S. *Immunogenetics* **17**, 553-596 (1983).
18. Graff, R. J. in *Origins of Inbred Mice* (ed. Morse, H. C. III) 667-687 (Academic, New York, 1979).
19. Krauspe, R. & Scheer, A. *Analyt. Biochem.* **153**, 242-250 (1986).
20. Rüsche, E., Kuon, W. & Hämmerling, G. J. *Transplant. Proc.* **15**, 2093-2096 (1983).
21. Lemke, H. Hämmerling, G. J. & Hämmerling, U. *Immunol. Rev.* **47**, 175-206 (1979).

ACKNOWLEDGEMENTS. We thank Jan Klein for his continuous support, Peter Bohley for discussions and for submitting H.J.W.'s Diplomarbeit to the faculty, Thomas Schechinger for help in setting up the HPLC system, Günther Jung and Karl-Heinz Wiesmüller for synthetic peptides, Stefan Stevanović for the sequencing attempt, Stefan Faath for technical assistance, Hansjörg Schild for reading and Petra Wiedmann for preparing the manuscript. This work was supported by Sonderforschungsbereich 120.

Identification and isolation of a membrane protein necessary for leukotriene production

Douglas K. Miller*, John W. Gillard†, Philip J. Vickers‡, Sharon Sadowski*, Claire Léveillé‡§, Joseph A. Mancini‡, Peter Charleson†, Richard A. F. Dixon||, Anthony W. Ford-Hutchinson‡, Réjean Fortin†, Jacques Yves Gauthier†, John Rodkey||, Ronda Rosen*, Carol Rouzer‡§, Irving S. Sigal*||, Catherine D. Strader* & Jilly F. Evans‡

* Department of Biochemistry and Molecular Biology, Merck Institute for Therapeutic Research, PO Box 2000, Rahway, New Jersey 07065, USA
† Departments of Medicinal Chemistry and ‡ Pharmacology, Merck Frosst Centre for Therapeutic Research, PO Box 1005, Pointe Claire-Dorval, Québec H9R 4P8, Canada

|| Department of Molecular Biology, Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486, USA

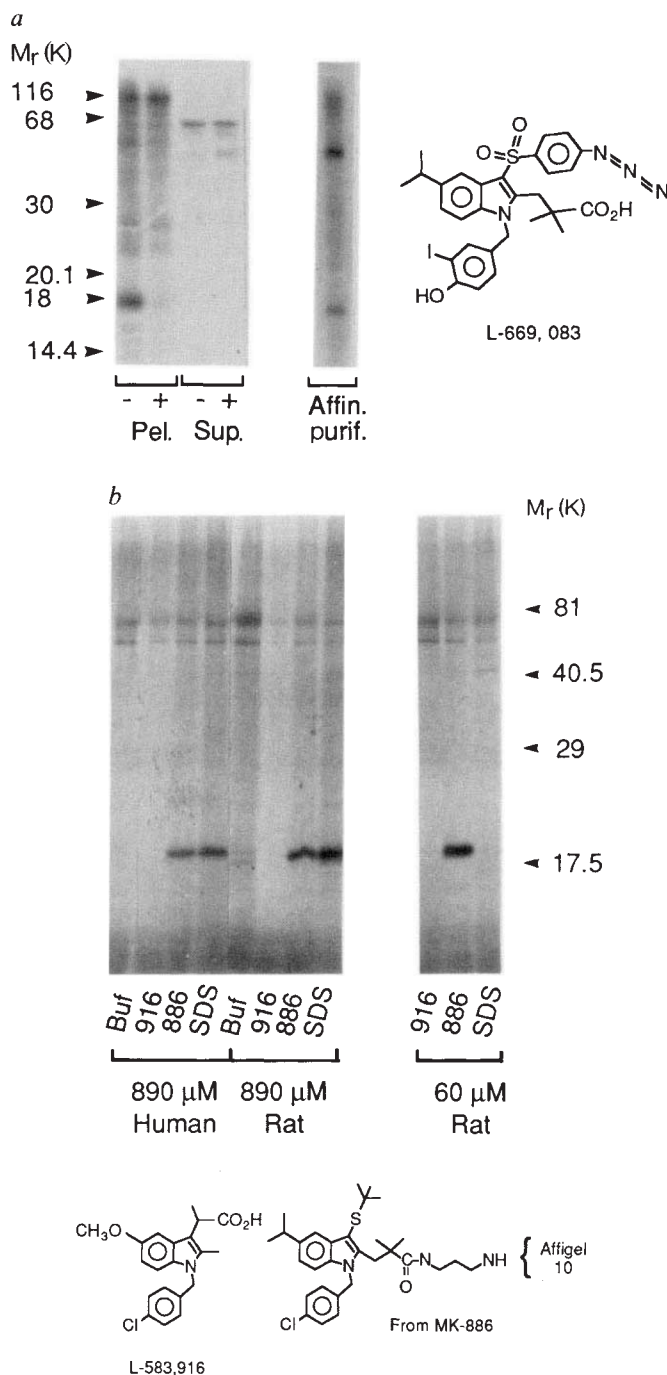
SEVERAL inflammatory diseases, including asthma, arthritis and psoriasis are associated with the production of leukotrienes by neutrophils, mast cells and macrophages¹⁻³. The initial enzymatic step in the formation of leukotrienes is the oxidation of arachidonic acid by 5-lipoxygenase (5-LO) to leukotriene A₄ (refs 4-6). Osteosarcoma cells transfected with 5-LO express active enzyme in broken cell preparations, but no leukotriene metabolites are produced by these cells when stimulated with the calcium ionophore A23187, indicating that an additional component is necessary for cellular 5-LO activity⁷. A new class of indole leukotriene inhibitor has been described that inhibits the formation of cellular leukotrienes but has no direct inhibitory effect on soluble 5-LO activity⁸. We have now used these potent agents to identify and isolate a novel membrane protein of relative molecular mass 18,000 which is necessary for cellular leukotriene synthesis.

The cellular target of the indole leukotriene inhibitor MK-886 (Fig. 1) was identified by its specific labelling with an ¹²⁵I radio-labelled photoaffinity probe (¹²⁵I-L-669,083) and by its retention on agarose gels to which analogues of MK-886 had been bound.

§ Present addresses: Immunology Laboratory, École de Médecine Dentaire, Université Laval, Québec 61K 7P4, Canada (C.L.) and Department of Chemistry, Western Maryland College, Westminster, Maryland 21157, USA (C.R.).

† Deceased.

FIG. 1 SDS-polyacrylamide gel electrophoresis of neutrophil cell extracts labelled by ^{125}I -L-669,083 (a) or eluted from MK-886 affinity columns (b). a, Inhibition of photoaffinity labelling of rat neutrophil 100,000g centrifugation pellet (Pel.) or supernatant (Sup.) fractions. In lanes designated (-) and (+), labelling was respectively in the absence or presence of 100 nM MK-886 (L-663,536; 3-[1-(*p*-chlorophenyl)-5-isopropyl-3-tert-butylthio-1H-indol-2-yl]-2,2-dimethylpropanoic acid) (left-hand four lanes). The far right lane shows the migration of MK-886 affinity-purified (Affin. purif.) human neutrophil proteins that had been labelled with ^{125}I after elution from the column (Fig. 1b). b, Silver-stained SDS-polyacrylamide gels of CHAPS detergent-solubilized human or rat neutrophil microsomal proteins that had been released from MK-886-coupled affinity columns (890 or 60 μM). Release of rat microsomal proteins by four column volumes of buffer (Buf), 100 μM L-583,916 (916), 100 μM MK-886 (886), or 0.1% SDS. The relative effectiveness of release of the 18K protein from the columns by MK-886 corresponded to the concentration of the bound MK-886; from the gels in which MK-886 was bound at high concentrations (890 μM), the 18K protein was released only partially by 100 μM MK-886, whereas at 60 μM affinity-bound MK-886, all of the protein was released. $M_r \times 10^{-3}$ values are indicated. METHODS. Rat peritoneal neutrophils¹³ or human peripheral neutrophils¹⁴ at 10^8 cells ml^{-1} were sonicated in 50 mM Tris-HCl buffer, pH 7.4, 140 mM NaCl, 2 mM EDTA, 1 mM DTT, 10% glycerol (homogenization buffer), together with the protease inhibitors 1 mM phenylmethylsulphonyl fluoride, 2 mg ml^{-1} pepstatin, 2 mg ml^{-1} leupeptin, and 10 mM furyl saccharine. After removal of 1000g and 10,000g pellets, the 100,000g pellet was resuspended (3 mg ml^{-1}) in the homogenization buffer. For photoaffinity labelling, 100- μl aliquots of cellular fractions (1 mg ml^{-1}) were preincubated with or without MK-886 (in 1 μl dimethyl sulfoxide) for 2 min at 37 $^\circ\text{C}$, incubated with ^{125}I -L-669,083 (400,000 c.p.m.; 500–1,000 Ci mmol^{-1}) for 5 min at 37 $^\circ\text{C}$, photolysed for 2 min with a 450 W lamp suspended 15 cm above the plate, separated by SDS-PAGE (17–27% gradient gel), and autoradiographed for 2 days. For affinity-column fractionation, the microsome suspension was solubilized on ice for 30 min in 50 mM Tris-HCl buffer, pH 7.4, with 140 mM NaCl, 0.5 mM DTT, 5% glycerol and 1% CHAPS detergent. After centrifugation for 10 min at 30,000g, the supernatant was applied to 3-ml columns (6×10^9 cell equivalents per column) containing the indicated affinity gel (prepared by coupling the compound in tetrahydrofuran to preactivated Affi-Gel 10, capping with ethanolamine, and washing with isopropanol). The gels were washed ~150 ml solubilization buffer for 16 h at 5 $^\circ\text{C}$ and eluted for 1 h with four column volumes of buffer with or without the indicated compounds or with 0.1% SDS in 10 mM Tris-HCl, pH 7.4. Samples were vacuum dialysed overnight at 5 $^\circ\text{C}$ with a 10 mM Tris-HCl, pH 7.4, 0.25 mM DTT buffer followed by SDS-PAGE (10–20% gradient gels) and visualization by silver staining. For ^{125}I labelling, a 25- μl aliquot of a vacuum-dialysed pooled protein fraction released by MK-886 from the affinity column was diluted with 125 μl of 58 mM Tris-HCl, pH 6.9, 77 mM NaCl containing 2 mCi ^{125}I . Chloramine T (5 μl , 2 mg ml^{-1}) was added for 90 s followed by sodium metabisulphite (10 μl , 8 mg ml^{-1}). The radioactive proteins were separated from free ^{125}I on a Sephadex G-15 column.



Incubation of ^{125}I -L-669,083 with neutrophil extracts followed by irradiation with ultraviolet light resulted in the labelling of several proteins; but labelling was only specifically inhibited by MK-886 in the case of a membrane protein of relative molecular mass (M_r) 18,000 (18K) (Fig. 1a). When solubilized extracts of neutrophil membranes (100,000g pellets) were chromatographed over affinity columns prepared from analogues of MK-886, an 18K protein found in both rat and human neutrophils was bound and was subsequently eluted by MK-886 (Fig. 1b). This protein co-migrated on SDS-polyacrylamide gels with the photoaffinity-labelled protein (Fig. 1a). The 18K protein comprised only a minor portion of the 10,000g pellet protein and was essentially absent from other cellular fractions (data not shown). The photoaffinity labelling of the 18K protein was inhibited by MK-886 in a concentration-dependent manner comparable to the inhibition of leukotriene synthesis by this com-

pound (Fig. 2a): the 50% inhibitory concentration (IC_{50}) for photoaffinity labelling by MK-886 was 80 nM, whereas it is 100 nM (ref. 9) for inhibition of leukotriene synthesis in human leukocytes at similar protein concentrations. Indole analogues that do not inhibit leukotriene biosynthesis, such as L-583,916, did not inhibit photoaffinity labelling of the 18K protein, nor did they elute this protein from affinity columns (Figs 1b and 2b). Several other compounds that have inhibitory effects on leukotriene production, including direct inhibitors of 5-LO, neither eluted the 18K protein from the affinity gels (Fig. 2b) nor inhibited photoaffinity labelling of the 18K protein (data not shown).

To purify the 18K protein, the fraction released from the affinity column by MK-886 was chromatographed on a Superose-12 column (Fig. 3a) followed by a subsequent separation on two TSK-3000 columns linked in tandem (Fig. 3b). After

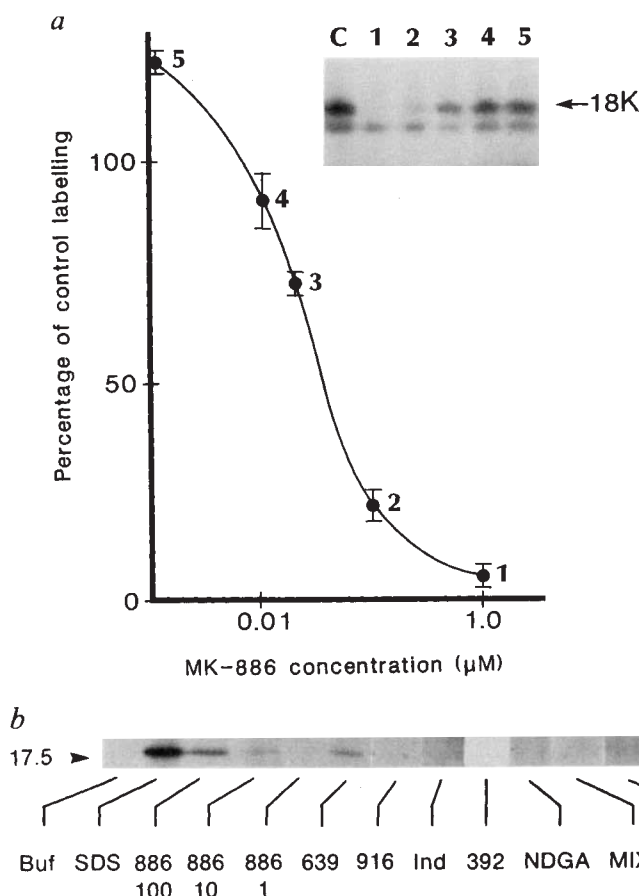
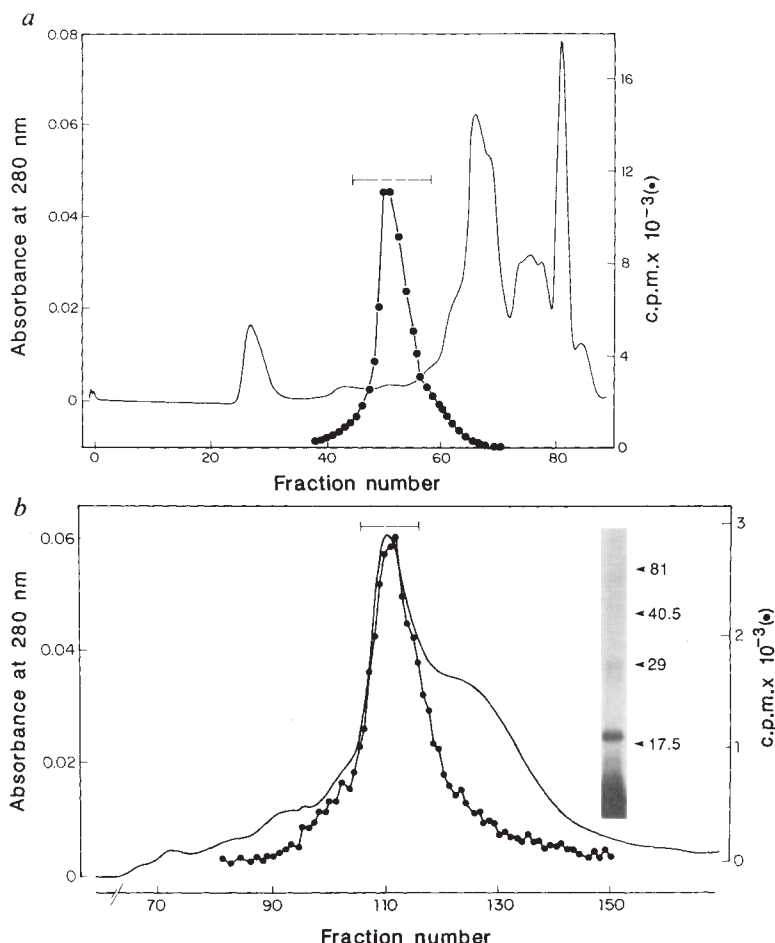


FIG. 3 Chromatographic separation of rat neutrophil proteins isolated from affinity columns. **a**, Absorbance at 280 nm tracing of rat microsomal proteins separated on a Superose-12 column. During the purification, the 18K protein was identified by SDS-PAGE or by co-chromatography with purified ^{125}I -labelled 18K protein. The bar represents the region combined for subsequent TSK-3000 chromatography. **b**, TSK-3000 chromatography of the pooled eluent obtained from the Superose-12 column showing absorbance and ^{125}I -labelled 18K protein. The bar indicates the pool of the 18K protein that was concentrated for reduction, alkylation and repurification on TSK-3000. The inset shows a silver-stained SDS-polyacrylamide gel of the protein isolated after the second TSK-3000 separation ($M_r \times 10^{-3}$ indicated). **METHODS.** Eluent from an L-665,210 affinity column, released with MK-886, was dried by speed-vac after vacuum dialysis, dissolved in 0.05% SDS-0.1 M NH_4HCO_3 , and separated on a Superose-12 column eluted with the same buffer at 0.25 ml min^{-1} . To determine whether 18K protein was present, 5- μl aliquots of the 0.25-ml fractions were analysed on 20% SDS-Phastgels (Pharmacia) followed by silver-staining. For TSK-3000 separation, two columns were linked in tandem and eluted with 0.05% SDS-0.1 M NH_4HCO_3 at 0.25 ml min^{-1} . The pooled fractions were concentrated and dried by speed-vac. For reduction and alkylation, the sample was suspended in 150 μl of a solution containing 3 μmol DTT in 67 mM Tris-HCl, pH 8.3, incubated for 6 h at 50°C , and alkylated for 1 h at 37°C , with 19 μmol iodoacetic acid brought to pH 8.3 with 1 M Tris base. DTT was then added to a final concentration of 1 M, and the sample incubated overnight at 37°C , dried, and rechromatographed on the TSK-3000 columns. After purification, the 18K protein was labelled with ^{125}I (see Fig. 1 legend) to provide a marker for subsequent purifications.

FIG. 2 Specificity of 18K-protein labelling by ^{125}I -L-669,083 (**a**) and of 18K-protein release from an indole affinity gel (**b**). **a**, Concentration-dependent inhibition of ^{125}I -L-669,083 labelling of the 18K protein in human leukocyte membranes by MK-886. Concentrations of MK-886 were 1 μM (1), 100 nM (2), 25 nM (3), 10 nM (4) and 1 nM (5). The band intensity of the 18K protein relative to a nonspecifically labelled protein in each lane migrating immediately following the 18K protein was measured by densitometry and results are expressed as a percentage of control labelling (C). Each point gives the mean and range of duplicate assays. **b**, Elution of rat membrane proteins from an L-665,210 (3-[1-(*p*-chlorobenzyl)-5-isopropyl-3-phenylsulphonyl-1H-indol-2-yl]-2,2-dimethylpropanoic acid; an active analogue of MK-886) affinity gel by several compounds with inhibitory effects on leukotriene production: the 5-LO inhibitor, L-651,392 (392)¹⁵; the antioxidant, nordihydroguaiaretic acid (NDGA); the cyclo-oxygenase inhibitor, indomethacin (Ind); the phosphodiesterase inhibitor, methylisobutyl xanthine (MIX); or the calmodulin antagonists W7, trifluoroperazine (TFP) and calmidazolium (Cal). The other incubations included buffer (Buf), 0.1% SDS, the active indole analogue L-654,639 (639; 3-[1-(*p*-chlorophenyl)-5-methoxy-3-methyl-1H-indol-2-yl]-2,2-dimethylpropanoic acid), or the inactive analogue L-583,916 (916). Unless otherwise indicated, the concentrations were 100 μM . The labelling of the 18K protein by the photoaffinity probe was also not inhibited by the following: MK-886 analogues showing no leukotriene inhibition; direct 5-LO inhibitors; eicosatetraynoic acid; thromboxane antagonists; dexamethasone, or disodium chromoglycate (data not shown). **METHODS.** Photoaffinity labelling was essentially as described in Fig. 1 legend using human leukocyte membranes pelleted at 100,000g. For the elution by different compounds from the affinity gel, 200 μl of a 50% slurry of the L-665,210 affinity gel that had been bound with CHAPS detergent-solubilized rat neutrophil membranes and then extensively washed, was centrifuged and the supernatant replaced with 250 μl of each of the compounds indicated dissolved in a buffer of 5 mM CHAPS, 10 mM Tris-HCl, 140 mM NaCl, 1 mM EDTA and 5% glycerol, pH 7.4. The gel was resuspended for 30 min at 5°C and centrifuged; the supernatant was lyophilized, then dissolved in SDS-PAGE sample buffer for electrophoresis followed by silver staining as described in the legend to Fig. 1b.



reduction and alkylation, the sample was repurified a second time over the TSK columns, resulting in a single protein peak as shown by silver staining after SDS-PAGE (Fig. 3b). The total amount of protein isolated from rat peritoneal neutrophils was $\sim 1.5 \mu\text{g}$ per 10^{10} cells. N-terminal sequence analysis of the purified rat 18K protein revealed the single hydrophobic sequence MDQEAVGNVLLAIVTLISVVQNAFFAXKVEL-ESKAQSG (single-letter amino-acid code). Cyanogen bromide cleavage of the purified 18K protein followed by fractionation over a C_4 microbore column identified two additional sequences:

SLAGILNHYLIFFFGSDFENY and XLFVXQKYFVGYL-GEXTQ, where X represents an unidentified amino acid. Tryptic cleavage of the 18K protein blotted onto nitrocellulose followed by separation on a C_8 microbore column revealed one unique sequence, XQSTPGYIFGKXIIILF. With over half of the predicted number of residues of the 18K protein contained within the four nonoverlapping peptides described above, there is little sequence homology of this protein to other known proteins.

A polyclonal rabbit antibody was prepared to the N-terminal 39 amino acids of the 18K protein. Immunoblots with this antibody on rat or human neutrophil 100,000g pellets or supernatants detected comparable amounts of a single 18K protein in the membrane fractions (Fig. 4a). Immunoblots also demonstrated the presence of this protein in membranes of a variety of leukocyte cell lines that make leukotrienes, whereas it was present in only trace or undetectable amounts in other cell lines that lack the ability to synthesize leukotrienes (D.K.M., unpublished results). When human leukocyte membranes labelled with the ^{125}I -L-669,083 photoaffinity probe were immunoprecipitated with the antipeptide antibody, a single labelled protein of M_r 18K was observed which was absent in membranes labelled in the presence of competing MK-886 (Fig. 4b). These results confirm that the protein identified by the photoaffinity probe is identical to that isolated by the affinity columns, and that the N-terminal sequence is derived from that protein.

In this report we have described the identification of a novel 18K membrane protein that interacts with a new class of leukotriene inhibitor exemplified by MK-886, but not with other agents known to inhibit leukotriene production. The observation that MK-886 inhibits cellular leukotriene formation in the absence of a direct effect on soluble 5-LO activity indicates that this 18K protein is a key component for cellular leukotriene biosynthesis. Because 5-LO is translocated from the cytosol to the membrane after activation^{10,9}, and MK-886 can inhibit and reverse that translocation⁹, the 18K protein may permit full activity of 5-LO in the membrane. Consequently, we have termed the 18K protein 'five-lipoxygenase-activating protein' (FLAP). FLAP could act directly to bind 5-LO to the membrane, or it could indirectly affect 5-LO activity. The observation that indoles that inhibit leukotriene synthesis do not inhibit arachidonic acid release in neutrophils (E. Ham and D. Soderman, unpublished results) indicates that FLAP is not a lipase. But because, unlike cyclo-oxygenase, 5-LO uses arachidonic acid only from phospholipid pools and not as the free lipid^{11,12}, FLAP could play an essential part in the transfer of arachidonic acid to 5-LO. The elucidation of the exact role that FLAP has in cellular leukotriene biosynthesis will lead not only to a greater understanding of the mechanism of this process but also to new therapeutic targets for inflammatory diseases. □

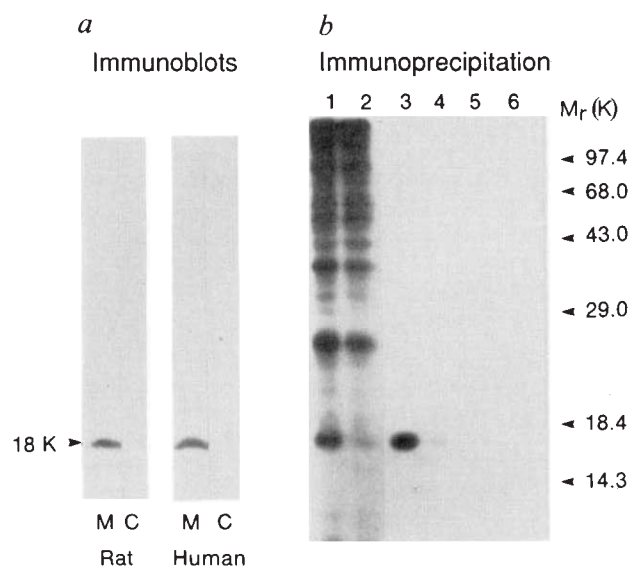


FIG. 4 Immunoblot analysis and immunoprecipitation of 18K protein labelled with ^{125}I -L-669,083. *a*, Immunoblots of rat and human neutrophil membrane (M) and cytosol (C) fractions using a rabbit antibody to the N-terminal peptide (residues 1–39 of the 18K protein). When preimmune serum was used, no 18K band was seen (data not shown). *b*, Autoradiograph of an SDS-polyacrylamide gel of human leukocyte membranes after ^{125}I -L-669,083 labelling in the absence (lanes 1, 3 and 5) or presence (lanes 2, 4 and 6) of $1 \mu\text{M}$ MK-886 (see Fig. 1 legend). Samples were either not immunoprecipitated (lanes 1 and 2), or immunoprecipitated with immune serum (lanes 3 and 4) or preimmune serum (lanes 5 and 6). $M_r \times 10^{-3}$ calibration is indicated.

METHODS. Rat and human neutrophil 100,000g pellet or supernatant samples ($20 \mu\text{g}$) were separated on 10–20% gradient gels, and blotted onto polyvinylidene difluoride membranes. After drying overnight the membranes were blocked with non-fat dry milk (50 mg per ml of PBS) for 1 h, 20°C , and with a subsequent 1-h incubation with a gelatin buffer (0.25% gelatin, 50 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 5 mM EDTA, 0.25% sodium azide, and 0.05% Triton X-100). The antipeptide antibody (prepared by a fusion of the DNA sequence coding for the peptide to the N terminus of the Cys Y protein, and its expression in and purification from *Escherichia Coli*¹⁶) was added at a 1:200 dilution in the gelatin buffer for 2 h. After washing three times, the membrane was incubated for 2 h with 5×10^5 c.p.m. ^{125}I -labelled protein A, washed five times, dried and exposed for autoradiography. For immunoprecipitation, after sonication of human leukocytes and removal of a 10,000g-centrifugation pellet, 800 μg of a 0–30% ammonium sulphate fraction containing membranes in 500 μl of 50 mM Tris-HCl, pH 8.0, 25 mM EDTA, 20% glycerol was incubated with ^{125}I -L-669,083 (2×10^6 c.p.m.) in the presence or absence of $1 \mu\text{M}$ MK-886, as in Fig. 2. The samples were mixed with 150 μl of 1% SDS, 100 mM Tris-HCl, pH 8.0 and incubated at room temperature for 15 min and at 95°C for 5 min. A buffer (0.5 ml) of 2% Triton X-100, 600 mM NaCl, 20 mM Tris-HCl, pH 7.2, was added, followed by incubation at 4°C for 30 min. The samples were then centrifuged at 15,000g for 15 min to remove precipitate. Immune or preimmune rabbit serum (50 μl) was added to the supernatants together with 4 mg protein A-Sepharose. After incubation for 1 h at 4°C , the samples were centrifuged and the pellets washed twice with Triton X-100-immunoprecipitation buffer and twice with 10 mM Tris-HCl, pH 7.0. The pellets were resuspended in electrophoresis sample buffer, heated and separated on 13.5% SDS-polyacrylamide gels. After drying, gels were autoradiographed.

Received 24 August; accepted 10 November 1989.

- Samuelsson, B. *Science* **220**, 568–575 (1983).
- Samuelsson, B., Dahlen, S. E., Lindgren, J. A., Rouzer, C. A. & Serhan, C. N. *Science* **237**, 1171–1176 (1987).
- Rokach, J. *Leukotrienes and Lipoxygenases* (Elsevier, Amsterdam, 1989).
- Shimizu, T., Radmark, O. & Samuelsson, B. *Proc. natn. Acad. Sci. U.S.A.* **81**, 689–693 (1984).
- Shimizu, T. et al. *Proc. natn. Acad. Sci. U.S.A.* **83**, 4175–4179 (1986).
- Rouzer, C. A., Matsumoto, T. & Samuelsson, B. *Proc. natn. Acad. Sci. U.S.A.* **83**, 857–861 (1986).
- Rouzer, C. A. et al. *J. biol. Chem.* **263**, 10135–10140 (1988).
- Gillard, J. et al. *Can. J. Physiol. Pharmacol.* **67**, 456–464 (1989).
- Rouzer, C. A. *J. biol. Chem.* (in the press).
- Rouzer, C. A. & Kargman, S. *J. biol. Chem.* **263**, 10980–10988 (1988).
- Kuehl, F. A., Jr., Dougherty, H. W. & Ham, E. A. *Biochem. Pharmacol.* **33**, 1–5 (1984).
- Chilton, F. H. *Biochem. J.* **258**, 327–333 (1989).
- Ham, E. A. et al. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4349–4353 (1983).
- Boyum, A. *Scand. J. Clin. Lab. Invest. (suppl. 97)* **21**, 77–89 (1968).
- Guindon, Y. et al. in *Advances in Prostaglandin, Thromboxane and Leukotriene Research* (eds Samuelsson, B., Paoletti, R. & Ramwell, P. W.) 554–557 (Raven, New York, 1987).
- Gan, Z. R. *Gene* **79**, 159–166 (1989).

ACKNOWLEDGEMENTS. We thank Guillermo Gimenez-Gallego and Vincent Strout for discussion and Morris Zimmerman for furoyl saccharine.

Requirement of a 5-lipoxygenase-activating protein for leukotriene synthesis

R. A. F. Dixon*, R. E. Diehl*, E. Opas†, E. Rands*, P. J. Vickers‡, J. F. Evans‡, J. W. Gillard§ & D. K. Miller||

Departments of *Molecular Biology, †Immunology and ||Biochemistry, Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486, and Rahway, New Jersey 07065, USA
Departments of ‡Pharmacology and §Medicinal Chemistry, Merck Frosst Centre for Therapeutic Research, PO Box 1005, Pointe Claire-Dorval, Québec H9R 4P8, Canada

LEUKOTRIENES, the biologically active metabolites of arachidonic acid, have been implicated in a variety of inflammatory responses, including asthma, arthritis and psoriasis^{1,2}. Recently a compound, MK-886, has been described that blocks the synthesis of leukotrienes in intact activated leukocytes, but has little or no effect on enzymes involved in leukotriene synthesis, including 5-lipoxygenase, in cell-free systems³. A membrane protein with a high affinity for MK-886 and possibly representing the cellular target for MK-886 has been isolated from rat and human leukocytes⁴. Here, we report the isolation of a complementary DNA clone encoding the MK-886-binding protein. We also demonstrate that the expression of both the MK-886-binding protein and 5-lipoxygenase is necessary for leukotriene synthesis in intact cells. Because the MK-886-binding protein seems to play a part in activating this enzyme in cells, it is termed the five-lipoxygenase activating protein (FLAP).

On the basis of the partial amino-acid sequence of purified rat FLAP⁴, we isolated cDNA clones 1 kilobase (kb) long from rat RBL-1 and human HL-60 cell-line cDNA libraries (Fig. 1). Both the rat and human cDNA clones encode proteins of 161 amino acids, which are 92% identical and contain all of the peptide sequences derived from purified rat FLAP. By hybridizing RNA from HL-60 cells with the cDNA, we identified a single 1-kb species (data not shown), indicating that the isolated clones are nearly of full length. Hydropathy analysis^{5,6} (data not shown) of the predicted rat or human FLAP amino-acid sequences demonstrated that the proteins are very hydrophobic, consistent with their membrane localization⁴. Three hydrophobic regions of 20–30 residues (Fig. 1) are predicted by hydrophobic-moment analysis⁷ (data not shown) to form membrane-spanning α -helices. On the basis of these data, a model for the structure of the protein can be proposed, in which the three transmembrane domains are connected by two hydrophilic loops, with the N terminus and C terminus on opposite sides of the membrane. Comparison of the sequence of FLAP with the sequences of other proteins revealed that although FLAP is not identical to any other known protein, it has a general similarity to several integral membrane proteins. This similarity seems to result from

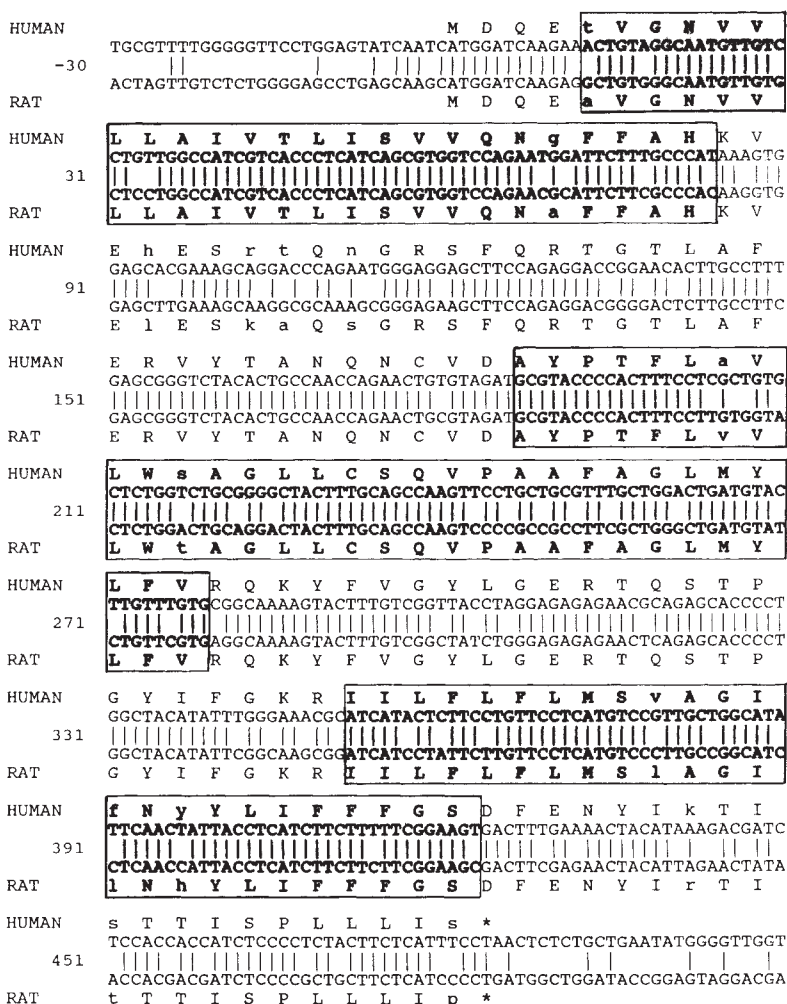
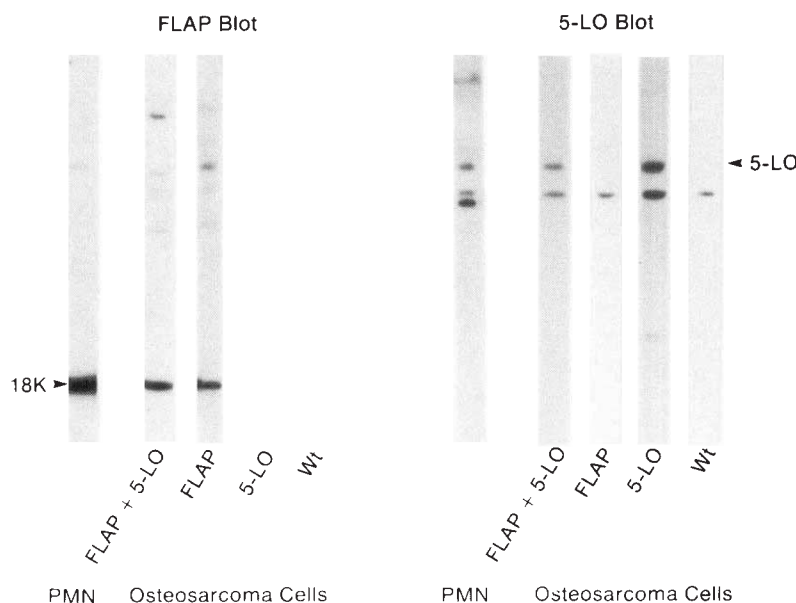


FIG. 1 Sequences of rat and human FLAP. A comparison of the nucleotide and predicted protein sequences (single-letter amino-acid code) of the rat and human FLAP open reading frames is shown. The complete sequence will be deposited with the EMBL database. Numbering begins with the first base of the initiation codon. Nucleotide identity is indicated by the vertical lines, amino-acid identity is indicated by capital letters for identical residues. The hydrophobic regions of the FLAP amino-acid sequence that are predicted to be membrane-spanning domains are indicated by shaded boxes. **METHODS.** All cells were grown in DME medium (GIBCO) containing 10% fetal bovine serum. HL-60 cells were differentiated by the addition of 1% dimethyl sulphoxide and 10 μ M dexamethasone and incubation at 37 °C for 5 days¹⁵. Poly(A)⁺ RNA was isolated from RBL-1 cells and differentiated HL-60 cells by the guanidinium isothiocyanate–CsCl method¹⁶, followed by oligo(dT) cellulose chromatography¹⁷. Double-stranded cDNA was prepared from the poly(A)⁺ RNA and ligated to λ ZAP arms (Stratagene) as described elsewhere^{15,18}. The DNA was packaged using Gigapack gold (Stratagene) and the resulting phage screened unamplified on *Escherichia coli* strain LE392 (refs 15 and 19). Pairs of complementary oligonucleotides with 5' extensions corresponding to a sequence capable of encoding each of the rat FLAP-derived peptide sequences (N terminus, MDQEA VGNV-LLAIVTLISVVQNAFFAKXVEESKAQSG; CNBr peptide fragments, YLFVXQKYFVGYLGEXTQS and SLA GILNHYLIFFG-SDFENY; tryptic peptide fragment, XQSTPPGYIFGKXILF)⁴ were synthesized using an Applied Biosystems 380A DNA synthesizer, annealed, radiolabelled using Klenow DNA polymerase and four ³²P-labelled dNTPs, and used to screen the RBL-1 cell cDNA library as described^{15,19}. Phage that hybridized to probes corresponding to all of the peptide sequences were purified and the inserts rescued according to manufacturer's instructions (Stratagene). The entire sequence of both strands of the cDNA inserts was determined as described^{15,20}. Nucleotide sequence analysis of several of the clones revealed a 161-amino-acid open reading frame after the first ATG in the sequence. All of the peptide sequences derived from purified rat FLAP were present in the predicted protein sequence. The open reading frame was followed by 408 base pairs of untranslated sequence ending in a poly(A) tail. The human FLAP cDNA was isolated by using the rat cDNA to probe the dimethylsulphoxide-differentiated HL-60 cell cDNA library. Several clones with inserts of 1 kb were isolated that contained an open reading frame similar to the one found in rat FLAP cDNA. Similarity searches were performed on the GenBank and EMBL databases using software produced by Intelligenetics.

FIG. 2 Expression of 5-LO and FLAP in transfected cell lines. Osteosarcoma 143 cell lines expressing human 5-LO, rat FLAP, or both (FLAP + 5-LO) were immunoblotted with anti-serum directed against either human 5-LO (5-LO blot) or rat FLAP (FLAP blot). Normal rat neutrophils (PMN) and parental osteosarcoma 143 cells (Wt) were also immunoblotted with the same antisera. The positions of the 80K human 5-LO protein and 18K rat FLAP are indicated by the arrows.

METHODS. Normal rat peritoneal neutrophils were prepared as described²¹. The isolation of osteosarcoma 143 cell lines expressing 5-LO has been previously described⁸. The expression vector used contains the cytomegalovirus immediate early promoter which drives the expression of the 5-LO gene, a hygromycin-resistance gene as a selectable marker, and the Epstein-Barr virus origin of replication⁸. The *EcoRI* fragment containing the cDNA insert for rat FLAP was cloned into the *EcoRI* site of the vector pSVLneo²². This vector contains the simian virus 40 origin of replication and uses the SV40 early promoter for expression of the FLAP gene. The vector also contains a neomycin-resistance gene as a selectable marker. The FLAP vector was transfected into osteosarcoma 143 cells that were previously transfected with the human 5-LO vector, and colonies resistant to both hygromycin and neomycin were isolated^{8,22}. The various cell lines were screened for expression of 5-LO and FLAP by immunoblotting with antibodies directed against each protein^{4,8}. Because the 5-LO vector is episomal in this cell line and has previously been found to be unstable⁸, the cell line expressing both 5-LO and FLAP was grown only in the presence of neomycin to allow for loss of the 5-LO gene. On passage, a revertant cell line was isolated that



retained expression of FLAP, but failed to express 5-LO. Immunoblotting of these cells was performed as previously described⁴. The origin and characterization of the antisera has also been described^{4,8}.

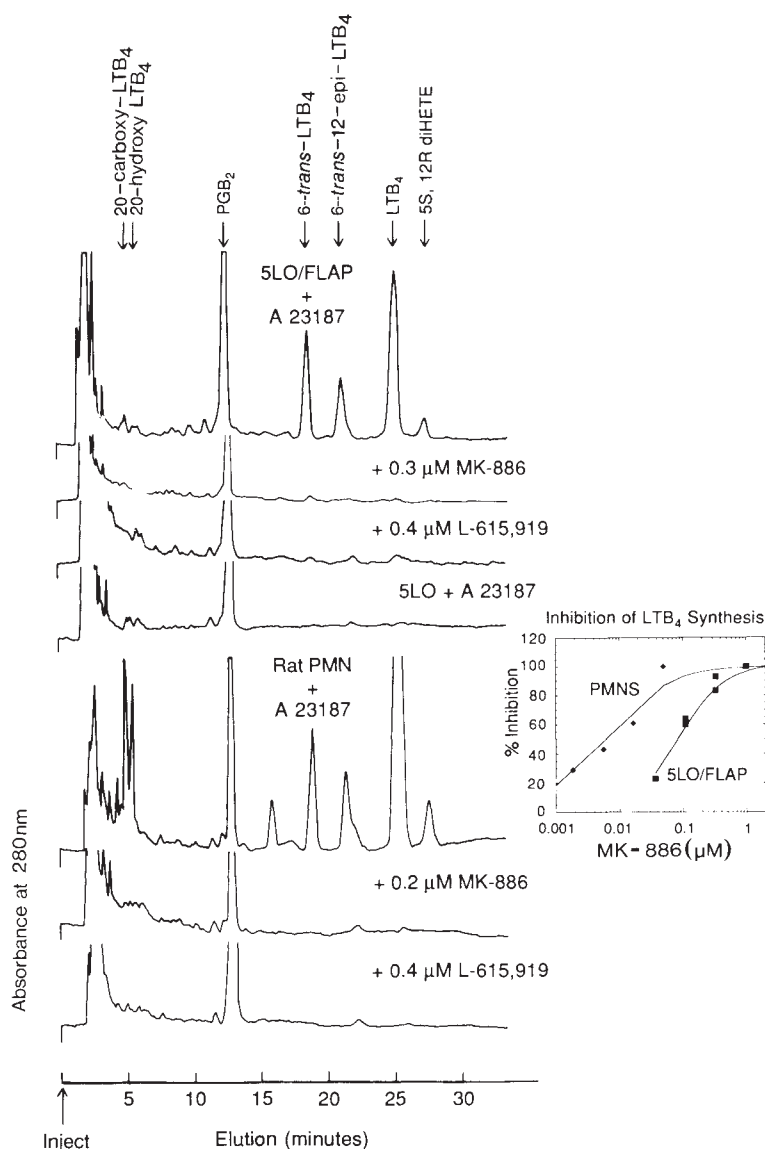


FIG. 3 Synthesis of leukotrienes by cells expressing 5-LO and FLAP. Osteosarcoma cells expressing 5-LO (5LO), 5-LO and rat FLAP (5LO/FLAP) or rat neutrophils (Rat PMN) were treated with the Ca^{2+} ionophore A23187 with or without the indicated compounds (MK-886 or L-615, 919), and 5-LO products were identified by reverse-phase HPLC. The top three absorbance profiles were of the osteosarcoma cells expressing both human 5-LO and rat FLAP, the fourth profile was of the osteosarcoma cell line expressing only human 5-LO, and the bottom three profiles were of rat neutrophils. The absorbance profiles of the parental osteosarcoma cell line and the cell line expressing only rat FLAP (data not shown) were identical to the absorbance profile of the cell line expressing only human 5-LO (fourth from top). The inset represents titrations of the inhibition of LTB_4 synthesis by MK-886 in the rat neutrophils (PMNs) or the osteosarcoma cell line expressing both 5-LO and rat FLAP (5LO/FLAP). Abbreviation: 5S,12R diHETE, 5(S),12(R)-dihydroxy-6-*trans*,8-*cis*,10-*trans*,14-*cis*-eicosatetraenoic acid.

METHODS. Confluent monolayers of osteosarcoma cells (1×10^7 cells) or a suspension of rat peritoneal neutrophils (1×10^8) were incubated with 4 ml Hank's PBS containing 15 mM HEPES buffer and $5 \mu\text{g ml}^{-1}$ A23187 for 10 min at 37°C . The extracellular fluids were removed, spiked with prostaglandin B_2 (PGB_2) and acidified with 150 μl acetic acid (1M). The samples were loaded onto a Sep-Pak C_{18} column (Waters, MA) washed with 15% methanol and with water, eluted with methanol, and dried. The samples were dissolved in 65% methanol, 35% H_2O , 0.05% acetic acid, 0.5 mM oxalic acid, pH 5.7, and isocratically separated by chromatography on a Supelcosil LC-18 column (25 cm $\times$ 4.6 mm, 5 μm bead size; Supelco, PA). The identity of each of the principal ultraviolet-absorbing species was confirmed by coelution with known standards and by spectral analysis. The identity of LTB_4 was also confirmed by radioimmuno assay. Where indicated, cells were pretreated with the concentration of the inhibitor shown for 5 min before stimulation by A23187. Relative leukotriene synthesis was determined by integrating the area under the LTB_4 peak, correcting for recovery using the PGB_2 internal standard, and normalizing to the level of leukotriene synthesis in the absence of inhibitor (100%). The 50% inhibitory concentration (IC_{50}) value for MK-886 inhibition is the concentration of drug that inhibits leukotriene synthesis by 50% (IC_{50} for PMN, 8 nM; IC_{50} for 5-LO/FLAP, 80 nM).

the hydrophobic residues in the putative transmembrane regions, rather than from any significant primary sequence identity. No consensus sequences for glycosylation, myristoylation or phosphorylation were identified in the FLAP sequence.

To determine whether FLAP is required for 5-lipoxygenase (5-LO) function in cells, we prepared human osteosarcoma 143 cell lines that expressed either FLAP or 5-LO alone, or both (5-LO/FLAP). Immunoblots of these cell lines with antisera directed against FLAP or 5-LO are shown in Fig. 2. As previously demonstrated, the 5-LO antisera recognized the 5-LO protein of relative molecular mass 80,000 (M_r 80K) and an unrelated 65K protein present in all cells⁸. The FLAP antisera recognized the 18K FLAP and some unrelated proteins of higher M_r (ref. 4). We were unable to detect either 5-LO or FLAP in parental osteosarcoma 143 cells, whereas both of these proteins were present in rat neutrophils. The level of 5-LO protein expressed in the transfected 5-LO or 5-LO/FLAP cells was comparable to or slightly higher than that observed in neutrophils. However, the amount of FLAP expressed in the transfected FLAP or 5-LO/FLAP cells was only 20% of that detected in neutrophils.

When the parental osteosarcoma 143 cells, or the cells expressing 5-LO or FLAP alone, were treated with the Ca^{2+} ionophore A23187, no arachidonic acid metabolites were detected (Fig. 3). By contrast, A23187 treatment of the cell line expressing both 5-LO and FLAP resulted in significant production of 5-LO products, including leukotriene (LT) B_4 and the LTA_4 hydrolysis products 6-*trans*- LTB_4 and 6-*trans*-12-*epi*- LTB_4 . A23187-treated rat neutrophils produced these products, as well as 5-HETE and the LTB_4 metabolites 20-hydroxy- LTB_4 and 20-carboxy- LTB_4 . The 5-LO/FLAP cell line produced only 14% of the neutrophil level of LTB_4 (0.15 nmol per mg protein versus 1.1 nmol per mg protein), whereas it produced 41% as much of the LTA_4 hydrolysis products (0.064 nmol 6-*trans*- LTB_4 and 0.059 nmol 6-*trans*-12-*epi*- LTB_4 per mg protein versus 0.18 nmol 6-*trans*- LTB_4 and 0.12 nmol 6-*trans*-12-*epi*- LTB_4 per mg protein). These data are consistent with the observation that the osteosarcoma cells contain only a small fraction of the amount of LTA_4 hydrolase that is present in the rat neutrophils (ref. 9, and data not shown). MK-886 and L-615,919 (a direct 5-LO inhibitor¹⁰) blocked leukotriene synthesis both in the 5-LO/FLAP cell line and in neutrophils (Fig. 3).

These experiments clearly demonstrate that the expression of FLAP, together with 5-LO, is essential for cellular leukotriene synthesis, and that the activity of the expressed protein is inhibited by MK-886. The mechanism by which FLAP activates 5-LO is not currently known. Because the translocation of 5-LO from the cytosol to the membrane after A23187 activation of neutrophils¹¹ is blocked by MK-886 (ref. 12), FLAP could be a membrane anchor for activated 5-LO. According to this model, a stable complex would be required to form at the membrane between activated 5-LO, FLAP and possibly other components of the leukotriene synthetic pathway, such as phospholipase A_2 or LTA_4 hydrolase. The formation of this complex could regulate the interaction of 5-LO with its substrate, arachidonic acid. Such a mechanism for 5-LO regulation is particularly intriguing, as other enzymes are translocated to the membrane upon Ca^{2+} activation^{13,14}. This type of protein-protein interaction could, therefore, represent a general mechanism for regulating enzymes that act on membrane-associated substrates. As such, proteins similar to FLAP could have a critical role in the activation of these other enzymes, and could serve as targets for the design of specific inhibitors of the pathways controlled by these enzymes. □

6. Kyte, J. & Doolittle, R. F. *J. molec. Biol.* **157**, 105–132 (1982).
7. Eisenberg, D., Schwarz, E., Kamaromy, M. & Wall, R. *J. molec. Biol.* **179**, 125–142 (1982).
8. Rouzer, C. A. et al. *J. biol. Chem.* **263**, 10135–10140 (1988).
9. Evans, J. F., Dupuis, P. & Ford-Hutchinson, A. W. *Biochem. biophys. Acta* **840**, 43–50 (1985).
10. Guindon, Y., Fortin, R., Lau, C. K., Rokach, J. & Yokim, C. European Patent 115394-A, *Bulletin* **84/32**, 08.08.84 (1984).
11. Rouzer, C. A. & Kargman, S. *J. biol. Chem.* **263**, 10980–10988 (1988).
12. Rouzer, C. A. et al. *J. biol. Chem.* (in the press).
13. Kraft, A. S. & Anderson, W. B. *Nature* **301**, 621–622 (1983).
14. Jaken, S. & Leach, K. L. *Ann. Rev. Med. Chem.* **23**, 243–252 (1988).
15. Dixon, R. A. F. et al. *Proc. natn. Acad. Sci. U.S.A.* **85**, 416–420 (1988).
16. Chargin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. *Biochemistry* **18**, 5294–5299 (1979).
17. Aviv, H. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1408–1412 (1972).
18. Dixon, R. A. F. et al. *Nature* **321**, 75–79 (1986).
19. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
20. Hattori, M. & Sakaki, Y. *Analyt. Biochem.* **152**, 232–238 (1983).
21. Ham, E. A. et al. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4349–4353 (1983).
22. Dixon, R. A. F. *Nature* **326**, 73–79 (1987).

ACKNOWLEDGMENTS. We thank S. Charleson for technical assistance, and Drs C. D. Strader, C. A. Rouzer, A. W. Ford-Hutchinson, C. Pickett, P. A. Anderson, E. M. Scolnick and I. S. Sigal for support.

Refined crystal structure of β -lactamase from *Citrobacter freundii* indicates a mechanism for β -lactam hydrolysis

C. Oefner, A. D'Arcy, J. J. Daly, K. Gubernator,
R. L. Charnas*, I. Heinze*, C. Hubschwerlen*
& F. K. Winkler†

Central Research Units and *Pharmaceutical Research Department,
F. Hoffmann-La Roche Co. Ltd, CH-4002, Basel, Switzerland

BETA-LACTAMASES (EC 3.5.2.6, 'penicillinases') are a family of enzymes that protect bacteria against the lethal effects of cell-wall synthesis of penicillins, cephalosporins and related antibiotic agents, by hydrolysing the β -lactam antibiotics to biologically inactive compounds. Their production can, therefore, greatly contribute to the clinical problem of antibiotic resistance^{1–4}. Three classes of β -lactamases—A, B and C—have been identified on the basis of their amino-acid sequence; class B β -lactamases are metalloenzymes, and are clearly distinct from members of class A and C β -lactamases⁵, which both contain an active-site serine residue involved in the formation of an acyl enzyme with β -lactam substrates during catalysis^{6–12}. It has been predicted that class C β -lactamases share common structural features with D,D-carboxypeptidases and class A β -lactamases, and further, suggested that class A and class C β -lactamases have the same evolutionary origin as other β -lactam target enzymes^{13,14}. We report here the refined three-dimensional structure of the class C β -lactamase from *Citrobacter freundii*^{12,15} at 2.0-Å resolution and confirm the predicted structural similarity. The refined structure of the acyl-enzyme formed with the monobactam inhibitor aztreonam at 2.5-Å resolution defines the enzyme's active site and, along with molecular modelling, indicates a mechanism for β -lactam hydrolysis. This leads to the hypothesis that Tyr 150 functions as a general base during catalysis.

We have determined the crystal structure of a chromosomally encoded class C β -lactamase of relative molecular mass 39,000 from the Gram-negative bacterium *Citrobacter freundii* 1203, by the multiple isomorphous replacement (MIR) technique. The structure has been refined to 2.0-Å resolution with a crystallographic *R*-value of 0.182 using the TNT software of Tronrud et al.¹⁶. The two subunits of the asymmetric unit were independently refined and their C_α positions can be superimposed with an r.m.s. difference of 0.59 Å. All active-site residues superimpose with an r.m.s. difference of 0.32 Å, which is comparable

† To whom correspondence should be addressed.

Received 24 August; accepted 10 November 1989.

1. Samuelsson, B. *Science* **220**, 568–575 (1983).
2. Samuelsson, B., Dahlen, S. E., Lindgren, J. A., Rouzer, C. A. & Serhan, C. N. *Science* **237**, 1171–1176 (1987).
3. Gillard, J. W. et al. *Can. J. Physiol. Pharmacol.* **67**, 456–464 (1989).
4. Miller, D. K. et al. *Nature* **343**, 278–281 (1990).
5. Hopp, T. P. & Woods, K. R. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3824–3828 (1981).

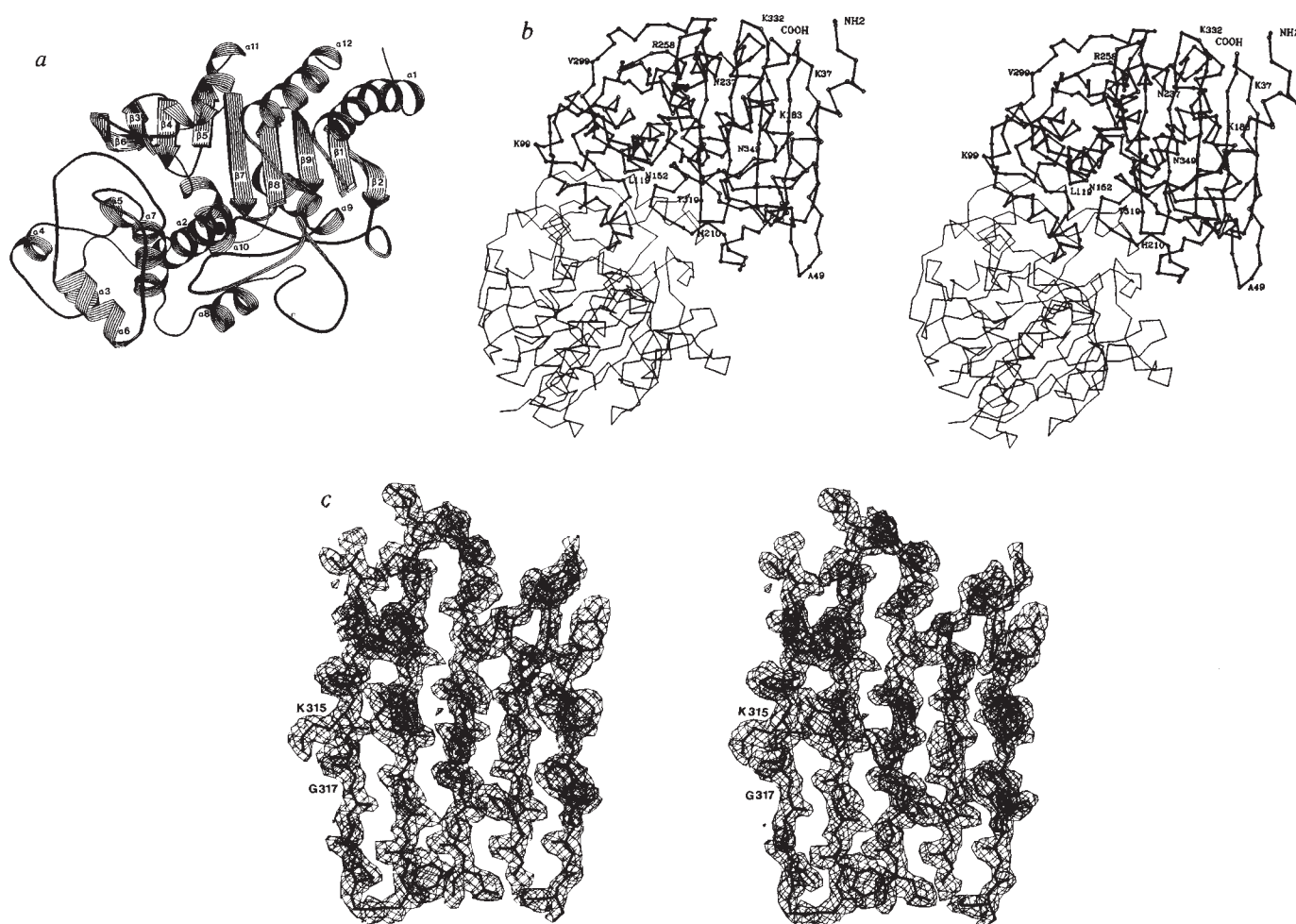


FIG. 1 Class C β -lactamase fold. *a*, Ribbon diagram³⁹ of the polypeptide chain of the class C β -lactamase from *C. freundii*. β -sheets (β 1– β 9) are represented by arrows, α -helices (α 1– α 12) by ribbons. The secondary-structure elements have been assigned for the refined coordinates using the algorithm of Kabsch and Sander⁴⁰ and are numbered sequentially through the amino-acid sequence. Their final assignments are: α 1, 5–23; α 2, 66–79; α 3, 89–93; α 4, 99–103; α 5, 106–110; α 6, 128–137; α 7, 152–163; α 8, 170–177; α 9, 227–238; α 10, 246–255; α 11, 280–292; α 12, 346–360; β 1, 27–34; β 2, 37–45; β 3, 258–262; β 4, 265–268; β 5, 272–275; β 6, 299–305;

β 7, 311–319; β 8, 322–329; β 9, 334–340 using the sequence numbering of Lindberg and Normark¹⁵. The position of the active-site serine residue Ser 64 is indicated. *b*, Stereo drawing depicting the α -carbon backbone of the dimeric β -lactamase structure. The view is slightly different from the one above. *c*, Stereo representation of the final omit map⁴¹ of the β -strands β 2, β 1, β 9, β 8 and β 7 contoured at 7 σ (σ is the r.m.s. electron-density fluctuation). The view is approximately the same as above, showing the position of the conserved triad Lys 315–Thr 316–Gly 317 (see text).

to the experimental error. The enzyme was purified over an aminophenylboronic acid column as previously described¹⁷, and then chromatographed over DEAE-Sephacel and CM-Trisacryl (20 mM triethanolamine-HCl, pH 7). After dialysis against 10 mM sodium-potassium phosphate, pH 7.0, the protein was crystallized at room temperature using standard vapour-diffusion techniques in hanging drops¹⁸, with 1.6 M sodium-potassium phosphate, pH 8.0, as the precipitating agent. Statistical information on data collection, MIR phasing, and structure refinement is summarized in Table 1.

A schematic view of the three-dimensional structure of the native class C β -lactamase and a skeletal drawing of the refined C_{α} positions is shown in Fig. 1. The enzyme is an α -helix/ β -sheet protein that is $\sim 60 \text{ \AA} \times 50 \text{ \AA} \times 40 \text{ \AA}$ in size. The three-dimensional structure is related to the class A β -lactamases from *Staphylococcus aureus*¹⁹ and *Streptomyces albus* G²⁰ and to the D,D-carboxypeptidase of *Streptomyces* R61²¹, despite poor sequence similarity. The enzyme can be described as a two-domain protein, with an interface that forms a crevice across the enzyme surface with the essential serine residue lying in the floor of the depression. The larger domain consists of a nine-

stranded antiparallel β -sheet flanked by three α -helices on one face and two α -helices on the other (for secondary structure assignments, see Fig. 1). The former three helices, α 1, α 12 and α 11, run antiparallel to each other in contrast to the corresponding helices in the class A enzymes. The β -sheet structure, with the topology β 2– β 1– β 9– β 8– β 7– β 5– β 4– β 3– β 6, contains the five-stranded sheet β 2– β 1– β 9– β 8– β 7 conserved in the related enzymes of known structure^{19–21}. Strands β 3– β 6 connect helices α 10 and α 11 and are only present in the class C enzyme. The smaller domain (residues Gly 63–Lys 224) contains seven α -helices (α 2– α 8) and extended loop regions. The essential serine residue, Ser 64 (ref. 15), is positioned at the N-terminal end of the buried central α 2-helix as in the class A β -lactamases and R61 carboxypeptidase. This residue is denoted as Ser 70 in the numbering scheme of Ambler for class A β -lactamases²² (the Ambler notation is indicated in the legend of Fig. 3).

Class A and C β -lactamases, D-Ala,D-Ala-carboxypeptidases and penicillin-binding proteins (PBPs) contain a few conserved regions (including the tetrad Ser-X-X-Lys, where X is any amino acid, and the triad Lys-Ser/Thr-Gly) and could be evolutionarily related as part of a superfamily of penicillin interactive

TABLE 1 Data collection and refinement

Space group: P ₂ ₁ 2 ₁ 2 ₁ (<i>a</i> =98.1 Å, <i>b</i> =84.6 Å, <i>c</i> =89.8 Å; two molecules per asymmetric unit)				
Statistics of data collection				
Derivative	Native	APM	BAI	Aztreonam
No. of measurements (no. of crystals)	113,118 (3)	58,062 (1)	53,355 (1)	72,200 (2)
No. of unique reflections (completeness (%))	46,181 (90)	17,299 (98)	22,924 (90)	22,927 (84)
Resolution limit (Å)	2.0	2.8	2.5	2.5
<i>R</i> _I *	0.07	0.04	0.04	0.09
MIR p.r.r. 10.0–3.8 Å (APM and BAI)				
SIR p.r.r. 3.8–3.0 Å (APM only)				
r.m.s. <i>F</i> _h / <i>E</i> †		2.1/1.8	1.2/0.8	
(all reflections/highest resolution range)				
<i>R</i> _C		0.57	0.66	
Mean figure of merit	0.65 for 13,658 reflections			
Final refinement parameters				
	Native	Aztreonam		
No. of cycles	320	119		
No. of atoms (non-hydrogen)	6,680	5,735		
No. of water molecules	1,070	79 (in active-site regions only)		
r.m.s. deviation of bond length (Å)	0.024	0.027		
r.m.s. deviation of bond angles (degrees)	2.5	2.8		
Resolution (Å)	6.0–2.0 Å	10.0–2.5 Å		
No. of <i>F</i> _{obs}	44,634	22,071		
<i>R</i> -factor	0.182	0.186		

Abbreviations: p.r.r., phasing in resolution range; obs, observed; calc, calculated. Initial screening of derivatives was carried out with low-resolution data (to 6.0 Å) measured on a four-circle Nicolet R3 diffractometer. The first derivative solved from its isomorphous difference Patterson was obtained by soaking native crystals in soaking buffer S (2.0 M Na⁺, K⁺-phosphate, pH 8.0) at a concentration of 1 mg ml⁻¹ PtBr₄ for 40 h. Four main sites were identified and could be grouped into two pairs related by an approximate non-crystallographic diad. Phases obtained from the isomorphous and anomalous differences of this derivative were used to solve additional derivatives by difference Fourier methods at low resolution. The two derivatives actually used for phasing were obtained by soaking the crystals in buffer S containing 1 mg ml⁻¹ *p*-aminophenyl mercuric acid (APM) and 0.5 mg ml⁻¹ of an iodinated boronic-acid compound (BAI), a reversible active-site inhibitor, respectively. The high-resolution data were collected on a Siemens/Nicolet area detector installed on an Elliot GX-21 rotating anode generator operating at 40 kV, 80 mA with a graphite monochromator. All crystals were cooled to ~4 °C during the measurement. Data frames of 0.1° with exposure times between 60 to 120 s were collected. The data were processed with the XENGEN software package³⁵. All subsequent crystallographic calculations were performed using the CCP4 computing package obtained from the Daresbury laboratory, England. The 10 mercury and 2 iodine sites, located in the Pt-phased difference Fourier maps could all be grouped into pairs, related by the noncrystallographic diad. Final heavy-atom parameters used for phasing were obtained by a Dickerson-type refinement³⁶ as implemented in the CCP4 program PHASE. The anomalous differences of the mercury derivative were included in the phasing. The quality of the resulting electron-density map was further improved by exploiting the noncrystallographic symmetry in conjunction with solvent flattening techniques. Using the Brice algorithm³⁷, five cycles of density modification and phase combination were run and an almost complete model could be built into the resulting 3.0-Å electron-density map. The missing segment (residues 195–210) was clearly defined in a 2*F*_{obs}–*F*_{calc} difference density obtained after a few cycles of TNT refinement. Binding studies with the monobactam aztreonam were performed by soaking native crystals of the *C. freundii* enzyme in buffer S at a concentration of 1.0 mM inhibitor. By monitoring the decrease of the aztreonam-induced X-ray intensity differences as a function of time in trial experiments, the half-life of the acyl-enzyme complex was estimated to be >30 h at 0 °C in the crystalline state³⁸. (Galleni *et al.* measured the deacylation rate of aztreonam with the β-lactamase from *C. freundii* OS61 (at pH 8.2 in 10 mM HEPES buffer containing 200 mM NaCl) by two different methods and derived values for half-life of the acyl enzyme of 36 and 57 min. By contrast, the half-life of the aztreonam acyl-enzyme complex formed from the *C. freundii* 1203 enzyme (as obtained from measurements of the regain of activity at 37 °C in 100 mM sodium phosphate, pH 7.0) was 110 ± 30 min. An extrapolation of the data from the 1203 enzyme predict that the deacylation rate at 0 °C could be 8–16 times slower. Data for the enzyme–aztreonam complex were collected at 0 °C from two crystals each exposed for 20 h.

* $R_i = \sum |I - \langle I \rangle| / \sum \langle I \rangle$.

† *F_h* heavy-atom-structure factor; *E_i* residual lack of closure.

‡ Cullis *R*-factor = $\sum |F_{h(obs)} - F_{h(calc)}| / \sum F_{h(obs)}$.

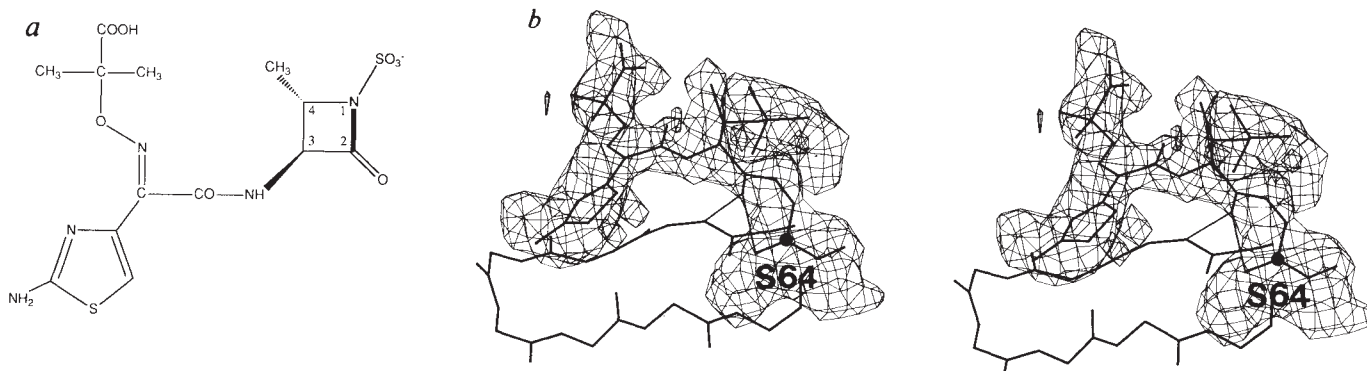


FIG. 2 Covalent structure and atom numbering of the monobactam aztreonam (a) and its ester-linked acyl-enzyme complex with Ser 64 of the class C β-lactamase from *C. freundii* (b). The complexed structure, refined at 2.5-Å resolution, is superimposed on the final 2*F*_{obs}–*F*_{calc} map contoured

at 12% of the maximum density, and part of the mainchain of β-strands 7 and 8 is shown. The observed donor–acceptor distances of the acyl group's carbonyl oxygen with the oxyanion hole are indicated by thin lines.

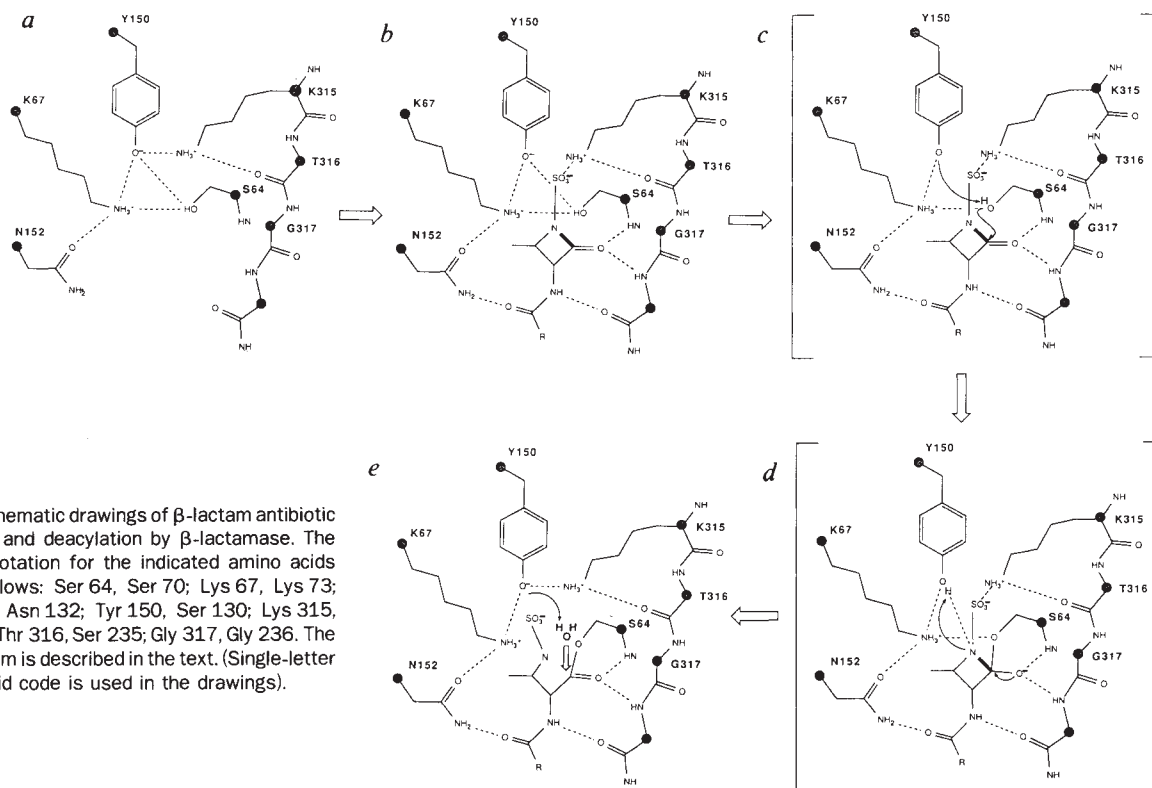


FIG. 3 Schematic drawings of β -lactam antibiotic acylation and deacylation by β -lactamase. The Ambler notation for the indicated amino acids is as follows: Ser 64, Ser 70; Lys 67, Lys 73; Asn 152, Asn 132; Tyr 150, Ser 130; Lys 315, Lys 234; Thr 316, Ser 235; Gly 317, Gly 236. The mechanism is described in the text. (Single-letter amino-acid code is used in the drawings).

enzymes¹³. In the *C. freundii* enzyme, the two conserved residues of the tetrad Ser 64 and Lys 67 are located on the same side of the α -2 helix, and the triad Lys 315–Thr 316–Gly 317 is part of β -strand 7 that delimits the active site. This is consistent with the suggestion²⁰ that these few conserved amino-acid residues are likely to be important in catalysis or substrate binding, or in both. The experimental data from the complex formed between the crystalline enzyme and the monobactam aztreonam provide further support for this hypothesis. The results (Fig. 2) show the presence of the expected Ser 64-linked acyl-enzyme intermediate and the opened ring β -lactam. As described for a class A β -lactamase²⁰, the carbonyl oxygen of the newly formed acyl group forms hydrogen bonds to the two NH groups of Ser 64 and Ser 318 in a way reminiscent of the oxyanion-hole hydrogen bonds of serine proteases of the trypsin family^{23,24}. The donor-acceptor distances observed in the refined complex at 2.5 Å resolution are 2.7 Å for Ser 64–NH and 2.9 Å for Ser 318–NH, respectively. The acyl-amino NH of the inhibitor forms a hydrogen bond with the carbonyl oxygen of Ser 318 and the portion of the antibiotic deriving from the β -lactam adopts a conformation that may be viewed as an extension of the β -sheet. Compared with the intact β -lactam, the opened-ring moiety shows rotations about the C3–C4 and N1–C4 bond of $\sim 70^\circ$ and 45° , respectively. The active site region of the native class C β -lactamase, including those residues likely to be important for catalysis, is shown schematically in Fig. 3a. Of particular interest is the remarkably dense hydrogen-bonding network involving both conserved lysine residues, the essential serine, Tyr 150 and Asn 152.

Following the example of Herzberg and Moulton¹⁹, we superimposed the native class C *C. freundii* enzyme onto trypsin (2PTC) using the active site serine and the oxyanion hole as reference points. A striking result of this superposition (data not shown) is that the phenolic oxygen of Tyr 150 is <0.5 Å from the $N_{\epsilon 2}$ position of the essential histidine in trypsin. This indicates that Tyr 150, as its anion, acts as a general base during the catalysis of β -lactam hydrolysis in a way similar to that of His 57 in trypsin²⁴. Because the experimental data shows that acylation causes only minor conformational changes within the active-site

region of native β -lactamase, the molecular modelling programs MOLOC and RIMG^{25,26} were used to investigate this possibility. Our models of the Michaelis complex, nucleophilic attack, tetrahedral intermediate formation, and collapse to give the acyl intermediate, are shown schematically in Fig. 3b–d. Tyr 150 is in an optimal position to act as the proton acceptor and donor in these reactions, whereas the N_{ϵ} of Lys 67 is too far away to donate a proton to the nitrogen expelled during collapse of the tetrahedral intermediate.

To use the same catalytic groups for deacylation, water must attack from the direction in which the β -lactam nitrogen is expelled. This requires a rotation about the C3–C4 bond to remove N1 from the path of the incoming water; it also removes the sulphonate from its interaction with Lys 315 and allows the initial hydrogen-bonding network to reform. Tyr 150 should therefore be less basic and consequently less effective as a general base in deacylation than in acylation, consistent with the observed rate-limiting deacylation¹⁰. (The constellation of the two positively charged lysines around Tyr 150 functions to keep it as an anion in solutions with pH values well below the normal pK_a of tyrosine (~ 10). The presence of adjacent positive charges causes large decreases in the pK_a 's of amino groups in proteins^{27–30}, tyrosine copolymers³¹, and in phenol-containing crown ethers complexed with ammonium ions³². The rate of proton abstraction by exogenously added amines in a genetically engineered aspartate transaminase decreases as the basicity decreases (Brønsted $\beta = 0.4$)³³. In addition, chlorination of the active-site tyrosine in the flavoenzyme D-amino acid oxidase decreases its basicity and decreases the rate of flavin reduction³⁴.)

Is this mechanism applicable to the other members of the superfamily? Tyr 150 in *C. freundii* β -lactamase could have a homologue in Tyr 159 of the R61 carboxypeptidase in which Lys 315 is replaced by histidine. A superposition of our data shows that the Tyr 150 in *C. freundii* β -lactamase corresponds to Ser 130 of the *S. aureus* enzyme (ref. 19; IBLM), a conserved residue in class A β -lactamases¹³; the spatial arrangement of the hydroxyl groups and the conserved lysines are equivalent. The participation of a serine anion as a general base catalyst in

an enzymatic acylation reaction is unprecedented and requires the active site region to be capable of lowering the serine pK_a substantially, similar to the proposal for the decrease of the pK_a of creatine ($pK_a = 14.3$) by creatine kinase³⁴. But the possibility of Lys 67 acting as the general base in the class A β -lactamases must also be considered. □

Received 25 September; accepted 23 November 1989.

- Sanders, C. C. & Sanders, W. E. Jr *Rev. Infect. Dis.* **5**, 639–648 (1983).
- Seeberg, A. H., Tolxdorff-Neutzing, R. M. & Wiedemann, B. *Antimicrob. Agents Chemother.* **23**, 918–925 (1983).
- Robert, M. & Falkow, S. *Nature* **266**, 630–631 (1977).
- Medeiros, A. A. *Br. med. Bull.* **40**, 18–27 (1984).
- Ambler, R. P. *Phil. Trans. R. Soc. B289*, 321–331 (1980).
- Jaurin, B. & Grundström, T. *Proc. natn. Acad. Sci. U.S.A.* **78**, 4897–4901 (1981).
- Knott-Hunziker, V., Orlek, B. S., Sammes, P. G. & Waley, S. G. *Biochem. J.* **177**, 365–367 (1979).
- Cohen, S. A. & Pratt, R. F. *Biochemistry* **19**, 3996–4003 (1980).
- Fisher, J., Charnas, R. L., Bradley, S. M. & Knowles, J. R. *Biochemistry* **20**, 2762–2731 (1981).
- Knott-Hunziker, V., Petrusson, S., Waley, S. G., Jaurin, B. & Grundström, T. *Biochem. J.* **207**, 315–322 (1982).
- Joris, B. *et al. Biochem. J.* **223**, 271–274 (1984).
- Sawai, T., Yamaguchi, A. & Tsukamoto, K. *Rev. Infect. Dis.* **10**, 721–725 (1988).
- Joris, B. *et al. Biochem. J.* **250**, 313–324 (1988).
- Samraoui, B. *et al. Nature* **320**, 378–380 (1986).
- Lindberg, F. & Normark, S. *Eur. J. Biochem.* **156**, 441–445 (1986).

- Tronrud, D. E., TenEyck, L. F. & Matthews, B. V. *Acta crystallogr.* **A43**, 498–501 (1987).
- Cartwright, S. J. & Waley, S. G. *Biochem. J.* **221**, 505–512 (1984).
- McPherson, A. *Meth. biochem. Analysis* **23**, 249–345 (1976).
- Herzberg, O. & Moul, J. *Science* **236**, 694–701 (1987).
- Dideberg, O. *et al. Biochem. J.* **245**, 911–913 (1987).
- Kelly, J. A. *et al. J. molec. Biol.* **209**, 281–295 (1989).
- Ambler, R. P. in β -Lactamases (eds Hamilton-Miller, J. M. T. & Smith, J. T.) 99–125 (Academic, New York, 1979).
- Kraut, J. A. *Rev. Biochem.* **46**, 331–358 (1977).
- Marquart, M., Walter, J., Deisenhofer, J., Bode, W. & Huber, R. *Acta crystallogr.* **B39**, 480–490 (1983).
- Müller, K. *et al. Trends in Medicinal Chemistry 88* (eds van der Goot, H., Dornay, G. Pallos, L. & Trimmerman, H.) 1–12 (Elsevier Science Publishers, Amsterdam, 1989).
- Müller, K. *et al. Bull. Soc. Chim. Belg.* **97**, 655–667 (1988).
- Schmidt, D. E. J. & Westheimer, F. M. *Biochemistry* **10**, 1249–1253 (1971).
- Quay, S. C. & Tronson, L. P. *Biochemistry* **22**, 700–707 (1983).
- Walsh, C. in *Enzymatic Reaction Mechanisms*, 674 (Freeman, San Francisco, 1979).
- Terwilliger, T. C., Weisman, L. & Eisenberg, D. *Biophys. J.* **37**, 353–359 (1982).
- Riordan, J. F. & Valle, B. L. *Meth. Enzym.* **25**, 515–521 (1972).
- Browne, C. M. *et al. J. Am. chem. Soc.* **107**, 2703–2712 (1985).
- Tonly, M. D. & Kirsch, J. F. *Science* **243**, 1485–1488 (1989).
- Rudie, N. G., Porter, D. J. T. & Bright, H. J. *J. biol. Chem.* **255**, 498–508 (1980).
- Howard, A. J. *et al. J. appl. Crystallogr.* **20**, 383–387 (1987).
- Dickerson, R. E., Weinzierl, J. E. & Palmer, R. A. *Acta crystallogr.* **B24**, 997–1001 (1968).
- Bricogne, G. *Acta crystallogr.* **A32**, 832–847 (1976).
- Galleni, M., Arnica, G. & Frère, J.-M. *Biochem. J.* **255**, 123–129 (1988).
- Priestle, J. P. *J. appl. Crystallogr.* **21**, 572–576 (1988).
- Kabsch, W. & Sander, C. *Biopolymers* **22**, 2577–2637 (1983).
- Bhat, T. N. *J. appl. Crystallogr.* **21**, 279–281 (1988).

ACKNOWLEDGEMENTS. We thank Dr D. W. Banner for help and advice throughout this project.

Homology of a yeast actin-binding protein to signal transduction proteins and myosin-I

David G. Drubin*, Jon Mulholland*, Zhimin Zhu* & David Botstein*

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

IN yeast, the cortical actin cytoskeleton seems to specify sites of growth of the cell surface^{1,2}. Because the actin-binding protein ABP1p is associated with the cortical cytoskeleton of *Saccharomyces cerevisiae*, it might be involved in the spatial organization of cell surface growth³. ABP1p is localized to the cortical cytoskeleton and its overproduction causes assembly of the cortical actin cytoskeleton at inappropriate sites on the cell surface, resulting in delocalized surface growth. We have now cloned and sequenced the gene encoding ABP1p. ABP1p is a novel protein with a 50 amino-acid C-terminal domain that is very similar to the SH3 domain in the non-catalytic region of nonreceptor tyrosine kinases (including those encoded by the proto-oncogenes *c-src* and *c-abl*), in phospholipase $C\gamma$ and in α -spectrin. We also identified an SH3-related motif in the actin-binding tail domain of myosin-I. The identification of SH3 domains in a family of otherwise unrelated proteins that associate with the membrane cytoskeleton indicates that this domain might serve to bring together signal transduction proteins and their targets or regulators, or both, in the membrane cytoskeleton.

Genetic mapping of the gene encoding ABP1p (*ABP1*) to a previously unassigned locus on chromosome III between *thr4* and *cdc39* (12 centiMorgans from *cdc39*), indicates that *ABP1* is a novel yeast gene⁴.

The *ABP1* nucleotide sequence and deduced amino-acid sequence are shown in Fig. 1. ABP1p has a relative molecular mass of 65,500 (M_r 65.5K), is very hydrophilic, very acidic ($pI = 4.6$, net charge = -45), and has a high proline content (11.2%). The overestimation of the size of ABP1p (M_r of 85K instead of 65.5K) when deduced from SDS-PAGE mobility is

characteristic of proline-rich proteins⁵. We identified two short repeated amino-acid motifs, indicating that there is a genetic duplication in *ABP1* (Fig. 2). Each unit of the first repeat (Fig. 2a) seems itself to have resulted from a duplication.

By searching the protein-sequence data base, we identified a 50-amino-acid region at the C terminus of ABP1p that is strikingly homologous to the C terminus of myosin-I^{6,7} and to the SH3 domains in the noncatalytic regions of nonreceptor tyrosine kinases and phospholipase $C\gamma$ ^{8,9}, as well as in α -spectrin¹⁰ and the *v-crk* transforming protein⁹ (Fig. 3). The importance of the SH3 domain in *c-src* and *c-abl* proteins is indicated by the observation that deletion and insertion mutations in it can lead to oncogenic activation^{11–13}.

On the basis of sequence conservation, the myosin-I SH3 region seems to be the closest relative of the corresponding ABP1p sequence. Moreover, whereas SH3 regions can be located anywhere in proteins, the ABP1p and myosin-I SH3 regions are located at the C terminus. Also, the 137 amino acids immediately upstream of the SH3 regions have limited similarity between ABP1p and *Dictyostelium* myosin-I; both regions are rich in alanine (12%) and proline (18% and 21% for ABP1p and myosin-I, respectively). But the myosin-I upstream region is 21% glycine and is basic, whereas the ABP1p region is 2% glycine and is acidic. The identification of SH3-related regions in non-metazoan cells indicates that the domain arose early in evolution.

A feature common to proteins with SH3 regions is physical or functional association, or both, with the cortical actin cytoskeleton, a submembranous protein network important for the regulation of cell shape, cell adherence and cell movement. ABP1p, myosin-I, spectrin, p60^{v-src}, p120^{v-gag-abl}, and p90^{v-gag-yes}, p80^{v-gag-yes} are all associated with the membrane cytoskeleton^{3,14–17}. Other indications of cytoskeletal associations are that, when overexpressed, type IV *c-abl* protein is seen to associate with actin stress fibres (as well as with the nucleus)¹⁸, and that *v-src* protein associates with a detergent-insoluble cytoplasmic matrix¹⁹. Also, the cadherins, which colocalize with apical actin bundles in living and detergent-extracted cells²⁰, contain in their cytoplasmic domains²¹ a region related, although remotely, to SH3 regions (a homology noted by D. Woods, personal communication).

Functionally, SH3-containing proteins affect cell properties through action on the cortical cytoskeleton, as illustrated by the two examples below. First, changes in actin cytoskeleton organization and cell shape are observed within 15 min or *v-src* activation²². Many substrates of the *v-src* tyrosine kinase (p60^{v-src}) are components of the cortical cytoskeleton¹⁷. It is

* Present addresses: Department of Molecular and Cell Biology, 471 Life Sciences Addition, University of California, Berkeley, California 94720 (D.G.D.); Genentech Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080, USA (J. M. and D. B.); Department of Biochemistry and Pharmacology, Harvard Medical School, Boston, Massachusetts 02115, USA (Z.Z.).

a

158 P P V K K S F T P - S K S P A P V S K K - E P V K
378 P P P K K S E P T I - I S P K P F S K P Q E P V K
168 K S P A P V S K - K E P V K
388 I S P K P F S K P Q E P V

b

200 D D D W N E P E L K E R D
565 D D D W W L G E L - E K D
435 D D D W D D D E D - E

<i>ABPI</i>	(539)	AEYDYDAAEDNELTFVENDKII--NIEFVDDDWLW-GELEKDGSKGLFSPSNVY	(25)
<i>Di myoI</i>	(1060)	ALYDYDASSDTDELSFKEGD-I-I-FVQKNGDGGWTCGEL-KSQCGKWAPNTYL	(26)
<i>myoII</i>	(1097)	ALYDYDAQTGDELTFFKEGDTI--VHQDKPAGWWE-GEUN--GKRGWVPAANY	(25)
<i>myoIB</i>	(984)	ALYDFAAENPDELTFFNEGAVT-VINKSNPDWWE-GEL--NGQRGVFPASYV	(22)
<i>plc</i>	(798)	ALFDYKAQREDELTFTKSA-I-IQNVEQCGEGGW-RGD-YHHKKQLWFPSPNVY	(20)
<i>v-yes</i>	(372)	ALYDYEARITDDLSFKKGERFQ-I-IMNTEGDWEEA-RSIAFGTKGYIPSNVY	(18)
<i>syn</i>	(89)	ALYDYEARTEDDLSFHKHKEGFQ-I-IINSEGDWEEA-RSLTTGTGTGYIPSNVY	(18)
<i>hck</i>	(64)	ALYDYEAIHEDLSFQKGDQMV-VLEES-GEWWKA-RSLATRTKEGYIPSNVY	(17)
<i>v-src</i>	(88)	ALYDYESRTETDLSFKKGERLQ-IVNNTEGDWWLA-HSLTTGTGTGYIPSNVY	(17)
<i>lyn</i>	(70)	ALYPYDGHEDLSFHKHKEGMK-VLEF-HGEWWKA-KSLLTKEGFGIPSNVY	(16)
<i>c-src</i>	(102)	SLYDYKSRHSDLSFHKMGDRME-VIDDTESDWWRV-VNLTTRQEGILINLFV	(15)
<i>spectrin</i>	(974)	ALYDYQEKSPREVT-MKKGDILTLNLTNKDWKVVVEVND--RQ-GFVPAAYV	(15)
<i>Hu abl</i>	(86)	ALYDFVASGDNTLSITKGE-KLRVLGYNHNGEWC-EAQTKNGQ-GWVPSNYI	(15)
<i>Dr abl</i>	(211)	ALYDFQAGGENQLSLKKGE-QVRILSYNKGSEWC-EAHSDDSGNVGWVPSNYV	(14)
<i>v-crk</i>	(375)	ALYDFKPGNDGDLPFKKGD-I-ILKIRDPKEQGWNA-EDMDGKR-GMIVPVVY	(13)
<i>lsk</i>	(67)	ALHSYSEPHDGDGLGFEKGEOLR-I-LEOS-GEWWKA-OSLTLTGGEQGFIPFNV	(13)

significant that mutations in the SH3 region of p60^{v-src} can affect the shape of a transformed cell, indicating a direct role for this domain in the cell shape changes (reviewed in ref. 23). Second, rapid tyrosine phosphorylation of phospholipase C γ and the rapid stimulation of inositol 1,4,5-trisphosphate formation in cells treated with epidermal growth factor are associated with rapid changes in cell morphology, cytoskeleton organization and phosphorylation^{24,25}. Inositol 1,4,5-trisphosphate has been proposed to regulate the actin-binding proteins gelsolin, profilin and myosin-I^{14,26}. The SH3 domain might bring together signalling proteins and their targets or regulators, or both, in the membrane cytoskeleton by interaction with a common cellular ligand.

Because several of the proteins that contain SH3 domains bind to actin, it is possible that actin is an SH3 ligand. This possibility is supported by the location of the SH3 domain of myosin-I within a 250-amino-acid proteolytic fragment that binds to actin in an ATP-insensitive manner¹⁴. It is significant that brush-border myosin-I²⁷ lacks an SH3 domain and does not contain the ATP-insensitive actin-binding site. Also, biochemical studies indicate that actin can bind to p60^{v-src} and inhibit its tyrosine kinase activity²⁸. Although spectrin binds to actin, the actin-binding site is believed to be in a region other than the SH3 domain¹⁶. Other proteins should also be considered as candidates for SH3-binding ligands. For example, calpactin-I is a candidate because it binds to spectrin and is a substrate for p60^{v-src} (ref. 10). Another candidate is ezrin, because like spectrin and phospholipase C γ , it is phosphorylated rapidly when cells are treated with epidermal growth factor²⁵.

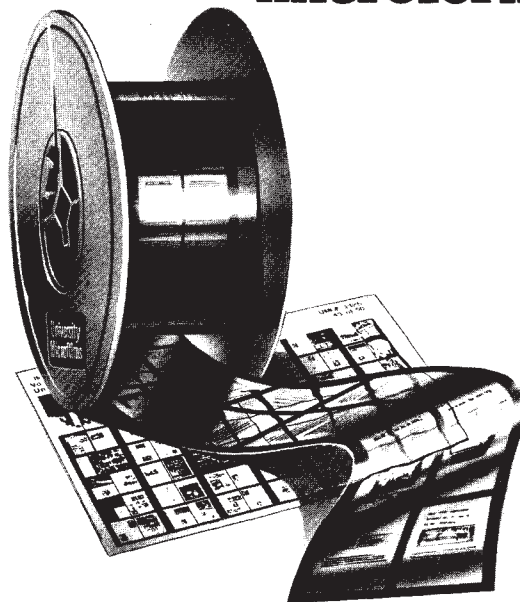
The identification of SH3-binding ligands and determination of whether the various SH3 domains have the same function are now required. The discovery of a protein with an SH3 domain in yeast should allow the use of classical and molecular genetic approaches to identify interacting proteins and to test the functional equivalence of different SH3 regions. The identification of new proteins with SH3 domains reported here indicates that more members of this protein family are likely to be discovered. $\square$

Received 12 October; accepted 14 December 1989.

- Adams, A. E. M. & Pringle, J. R. *J. Cell Biol.* **98**, 934-945 (1984).
- Novick, P. & Botstein, D. *Cell* **40**, 405-416 (1985).
- Drubin, D. G., Miller, K. G. & Botstein, D. *J. Cell Biol.* **107**, 2551-2561 (1988).
- Mortimer, R. K., Schild, D., Contopoulos, C. R. & Kans, J. A. *Yeast* **5**, (1989).
- Lee, G., Cowan, N. & Kirschner, M. *Science* **239**, 285-288 (1989).
- Jung, G., Korn, E. D. & Hammer III, J. A. *Proc. natn. Acad. Sci. U.S.A.* **84**, 6720-6724 (1987).
- Jung, G., Saxe III, C. L., Kimmel, A. R. & Hammer III, J. A. *Proc. natn. Acad. Sci. U.S.A.* **86**, 6186-6190 (1989).
- Stahl, M. L., Ferenz, C. R., Kelleher, K. L., Kriz, R. W. & Knopf, J. L. *Nature* **332**, 269-272 (1988).
- Mayer, B. J., Hamaguchi, M. & Hanafusa, H. *Nature* **332**, 272-275 (1988).
- Lehto, V.-P., Wasenius, V.-M., Salven, P. & Saraste, M. *Nature* **334**, 388 (1988).
- Kato, J.-Y. *et al. Molec. cell. Biol.* **6**, 4155-4160 (1986).
- Potts, W. M., Reynolds, A. B., Lansing, J. T. & Parsons, J. T. *Oncogene Res.* **3**, 343-355 (1988).
- Jackson, P. & Baltimore, D. *EMBO J.* **8**, 449-456 (1989).
- Adams, R. J. & Pollard, T. D. *Nature* **340**, 565-568 (1989).
- Fukui, Y., Lynch, T. J., Breska, H. & Korn, E. D. *Nature* **341**, 328-331.
- Morrow, J. S. *Curr. Op. Cell Biol.* **1**, 23-29 (1989).
- Burridge, K., Fath, K., Kelly, T., Nuckolls, G. & Turner, C. A. *Rev. Cell Biol.* **4**, 487-525 (1988).
- Van Etten, R. A., Jackson, P. & Baltimore, D. *Cell* **58**, 669-678 (1989).
- Hamaguchi, M. & Hanafusa, H. *Proc. natn. Acad. Sci. U.S.A.* **84**, 2312-2316 (1987).
- Hirano, S., Nose, A., Hattori, K., Kawakami, A. & Takeichi, M. *J. Cell Biol.* **105**, 2501-2510 (1987).
- Nagafuchi, A., Shirayoshi, Y., Okazaki, K., Yasuda, K. & Takeichi, M. *Nature* **329**, 341-343 (1987).
- Boschek, C. B. *et al. Cell* **24**, 175-184 (1981).
- Pawson, T. *Oncogene* **3**, 491-495 (1988).
- Wahl, M. I., Nishibe, S., Suh, P.-G., Rhee, S. G. & Carpenter, G. *Proc. natn. Acad. Sci. U.S.A.* **86**, 1568-1572 (1989).
- Bretscher, A. *J. Cell Biol.* **108**, 921-930 (1989).
- Matsudaira, P. & Janmey, P. *Cell* **54**, 139-140 (1988).
- Hoshimaru, M. & Nakanishi, S. *J. Biol. Chem.* **262**, 14625-14632 (1987).
- Tsen, S. D., Lee, R., Damiani, B., Hsieh, P. & Chen, L. B. *Cold Spring Harb. Symp. quant. Biol.* **44**, 967-974 (1980).
- Shtivelman, E., Lifshitz, B., Gale, R. P., Roe, B. A. & Canaani, E. *Cell* **47**, 277-284 (1986).
- Henkemeyer, M. J., Bennett, R. L., Gertler, F. B. & Hoffmann, F. M. *Molec. cell. Biol.* **8**, 843-853 (1988).

ACKNOWLEDGEMENTS. We thank Georjana Barnes, Tony Bretscher, Peter Bryant, Peter Jackson, Daphne Preuss, Steve Martin, Paul Matsudaira, James Trager, Ken Wertman and Dan Woods for discussions. This work was supported by the NIH and the American Cancer Society (D.B.) and a Helen Hay Whitney postdoctoral fellowship (D.D.).

nature is available in microform.



University Microfilms

International reproduces this publication in microform: microfiche and 16mm or 35mm film. For information about this publication or any of the more than 13,000 titles we offer, complete and mail the coupon to: University Microfilms International, 300 N. Zeeb Road, Ann Arbor, MI 48106. Call us toll-free for an immediate response: 800-521-3044. Or call collect in Michigan, Alaska and Hawaii: 313-761-4700.

☐ Please send information about these titles:

Name _____

Company/Institution _____

Address _____

City _____

State _____ Zip _____

Phone () _____

University
Microfilms
International

Solid-phase protein sequence analysis

R. Aebersold

The solutions to many protein analytical problems require flexible chemistries at a high sensitivity. Solid-phase sequence analysis is one way to eliminate limitations inherent in current methods.

THE scope of protein sequence analysis has changed in recent years from the aim of determining the complete primary structure of a protein by the sequence analysis of peptides, to being an essential partner in a biotechnology based upon both nucleic acid and protein chemistry.

To make this integrated approach even more powerful, protein chemistry has to realize three major improvements. First, improved sensitivity is required for the analysis of low-abundance proteins. An increase in sensitivity by two orders of magnitude will make the direct sequence analysis of proteins separated in analytical two-dimensional gels a general approach, and will therefore be an essential step towards an integrated protein database, summarizing the structural, functional and clinical information from each separated protein. Second, shorter cycle times are necessary to increase sample throughput, and third, the nature and sites of modified amino acids must be determined at high sensitivity. Several recent developments indicate that solid-phase sequence analysis has the potential to realize the expected improvements.

Sequencing methods

Most protein sequences are determined using the Edman degradation of polypeptides non-covalently immobilized in the cartridge of a gas-liquid phase protein sequenator¹. At present, gas-liquid phase sequence analysis allows the determination of protein sequences at the low picomole level with cycle times of about 45 minutes. The sensitivity of the overall sequencing process is limited by the UV detection of phenylthiohydantoin — the final products of the Edman degradation. Furthermore, the nature and sites of modified amino acids escape detection with this method.

Non-covalent substrate immobilization in the sequenator greatly restricts the flexibility of degradation chemistries that can be explored, and so prevents further improvements in sequencing protocols.

Conceptually, solid-phase sequence analysis, the Edman degradation of polypeptides covalently attached to insoluble solid supports, is a superior approach (Fig. 1). Permanent immobilization of the substrate eliminates most of the restrictions in chemical flexibility imposed by gas-liquid phase sequence analysis. In practice, however, the solid-phase approach, although pioneered almost twenty years ago, has not found wide-

spread use². Procedures for the covalent attachment of sequenceable polypeptides to solid supports were widely thought to be tedious, inefficient and not generally applicable. More importantly, the classical protein carriers — activated porous beads — were fundamentally incompatible with the most sensitive procedures for the isolation of proteins for sequence analysis.

New developments

As with any approach to sequencing polypeptides, the performance of solid-phase Edman degradation depends on compatible, sensitive and efficient methods for the preparation of substrates for sequencing, and on high-quality instrumentation. Recently, we and others have developed high-yield procedures for the preparation of proteins and their cleavage products for solid-phase sequence analysis at the low picomole and subpicomole level^{3,7}.

The most important development was the change in geometry of the activated support. Traditionally, macroscopic porous beads were used as solid-phase protein carriers⁸, and protein-carrier complexes were packed into small columns which served as reaction cartridges^{2,8,9}. We chose activated porous membranes or discs as carriers for several reasons. (1) The geometry of the activated membranes is compatible with the isolation of proteins separated by methods with the highest resolving power, that is, one- and two-dimensional polyacrylamide gel electrophoresis. Proteins separated by gel electrophoresis can be directly transferred and covalently attached to the membrane by electrophoretic transfer (electroblotting) in a single operation^{3,10}. Electroblotting is the method of choice for isolating proteins for high-sensitivity sequence analysis because of its simplicity, efficiency and speed. In an elegant variation of this procedure, electroblotted proteins are fixed to solid supports by entrapment in a thin polymer network^{5,7}. (2) The disc geometry optimizes coupling yields. Deposition of the polypeptides onto the disc by evaporation of the solvents ensures close proximity of re-

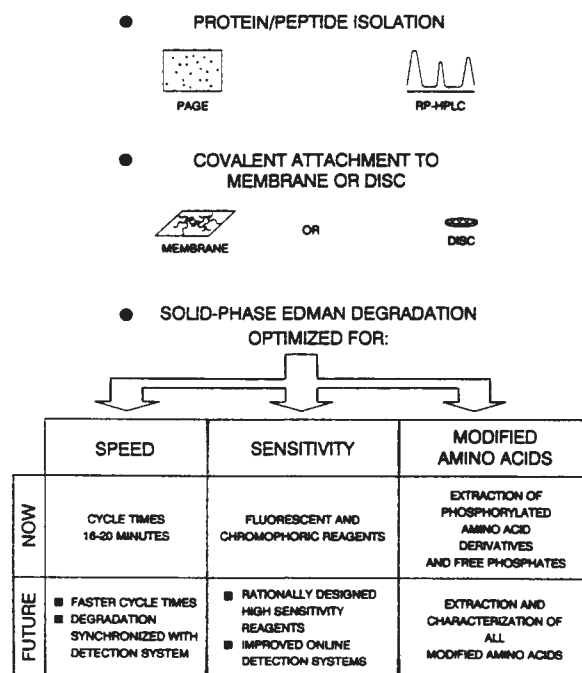


FIG. 1 The process of solid-phase protein sequence analysis. PAGE: polyacrylamide gel electrophoresis; RP-HPLC: reversed-phase high performance liquid chromatography.

active groups on the membrane with those in the polypeptide and, by greatly restricting diffusion, achieves the very high reagent/reactant concentrations favoured to drive the coupling reactions to completion^{4,6}. (3) The disc geometry permits rapid and efficient washing of the sample during the sequencing process, thus maximizing speed and minimizing the amount of chemical contaminants⁴. (4) The disc geometry drastically reduces substrate losses during transfer of the sample into the sequenator.

High sensitivity solid-phase Edman degradation of substrates immobilized on activated discs or membranes can be most efficiently performed on dedicated solid-phase sequenators. Such an instrument, the Model 6600 Pro Sequencer (MilliGen/Biosearch), has recently become commercially available. In principle, however, any modern protein sequenator, including gas-liquid phase machines, after undergoing limited hardware changes, will be compatible with advanced solid-phase sequencing protocols. Initial experiments performed on a prototype solid-phase sequenator at the California Institute of Technology have shown that significant improvements in sequencing sensitivity and speed could be combined with the ability to determine sites of phosphorylation at the low picomole level^{4,11,12}. These

protocols are now being adapted to commercially available sequencers.

Protein phosphorylation is one of the most important post-translational modifications that escapes high-sensitivity detection in current sequencing methods. Simple modifications in hardware and chemistry have led to the efficient extraction of anilinothiozolinylphosphotyrosine in the solid-phase sequencing mode. Phosphorylated serine and threonine residues have been determined by the simultaneous detection of extracted, beta-eliminated phosphate and the corresponding phenylthiohydantoin in the respective degradation cycles^{4,12}.

Using the same prototype instrument, sequencing with phenylisothiocyanate, cycle times as short as 16 minutes were achieved^{4,11}. This represents the shortest cycle time reported to date for any mode of chemical sequence analysis. Probably the most significant consequence of the chemical flexibility inherent in the solid-phase approach is the possibility of using a wide variety of sequencing reagents yielding amino acid derivatives with enhanced detectability. We have used the chromophoric reagent 4-*N,N*-dimethylaminoazobenzene-4'-phenylisothiocyanate, a well characterized protein sequencing reagent, as a model compound for this class of reagents^{4,13}. Although sensitivity at the subpicomole level was easily achieved, increased sensitivity by two orders of magnitude will require the synthesis of new sequencing reagents rationally designed for optimal detectability in improved analytical systems consisting of mass-spectrometers or detectors for laser-induced fluorescence, photothermal refraction, or chemiluminescence connected on-line to capillary electrophoresis or microbore chromatography systems¹⁴⁻¹⁶.

The development of simple, fast, sensitive and highly efficient methods for the preparation of polypeptides for high-sensitivity solid-phase sequence analysis and the introduction of a commercially available solid-phase sequencer have revived interest in this approach. In the future, the synthesis of sequencing reagents with enhanced detectability, and the on-line connection of highly sensitive analytical systems, will undoubtedly produce further increases in sequencing sensitivity. □

Ruedi Aebersold is Assistant Professor, Biochemistry, at the Biomedical Research Centre, 2222 Health Sciences Mall, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5. For more information, fill in reader service number 100. Ruedi Aebersold would like to thank Dr D. Teplow for his critical review of the manuscript.

- Hewick, R.M., Hunkapiller, M.W., Hood, L.E. & Dreyer, W.J. *J. Biol. Chem.* **256**, 7990-7997 (1981).
- Laursen, R.A. *Eur. J. Biochem.* **20**, 89-102 (1971).
- Aebersold, R., Pipes, G., Nika, H., Hood, L.E. & Kent, S.B.H. *Biochemistry* **27**, 6860-6867 (1988).

Bio/Tech roundup

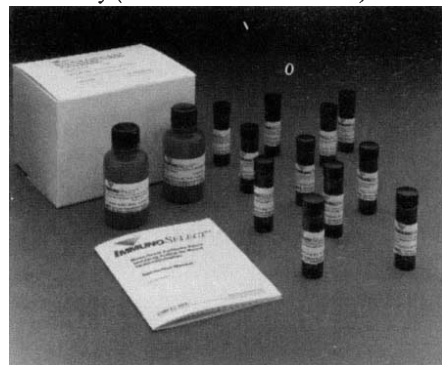
Products featured at next week's Bio/Technology Winter Symposium in Miami, Florida, include a high-throughput DNA sequencer and an antibody purification system.

PHARMACIA LKB Biotechnology announces the new Automated Laser Fluorescent (A.L.F.) **DNA sequencer**, which is designed to sequence and evaluate up to eight kilobases per day (*Reader Service No. 101*). The A.L.F. DNA Sequencer offers easy sample preparation and straightforward reaction chemistry, says Pharmacia. Using just one fluorescently-labelled primer, samples are prepared by classical Sanger methods with the sequencing kit. In less than one hour, samples can be loaded onto the gel, without the need for extra quantitation or precipitation steps. Custom fluorescent primers can be prepared using the primer synthesis kit. Sample bands are detected with a fixed laser beam that penetrates the entire width of the gel. Dedicated software, run on a multitasking computer system, offers complete system control, rapid interpretation of raw and processed data, post-run evaluation capabilities, and access to stored data while sequencing is in progress. See the A.L.F. DNA Sequencer in booths 31/32.

The System 100 biocompatible **affinity protein purification unit** provides a means of collecting purified antibody or antigen within 20 minutes, says Genex (*Reader Service No. 102*). Housed in a compact benchtop unit is a single-piston pump with rapid refill facility, a UV fixed-wavelength detector optimized for proteins at 280 nm, preprepared buffers in ready-to-use bottles, and GammaBind G silica or agarose columns — although other column matrices can be used. The system offers four modes of operation. In pro-

gram mode, the user can optimize methods by creating, storing and editing up to nine user-defined protocols. According to Genex, the System 100 is scalable and can be used to purify over one gram per day with 90-95 per cent purity. Stated applications include the purification of antibodies from serum and ascites, purification of antigens with immunoaffinity columns, and removal of IgG from serum for IgM or IgE detection. See Genex in booth 40.

Immunoselect is the new monoclonal antibody-based **isotyping system** for the identification of mouse immunoglobulins, without, says Life Technologies, cross reactivity (*Reader Service No. 103*). Based



Immunoselect isotyping system.

on an antibody-capture ELISA, Immunoselect utilizes monoclonal rat anti-mouse Ig antibodies against all the major classes and subclasses of mouse immunoglobulins. The isotyping system comes complete with all the necessary Gibco/BRL-brand reagents to isotype 50-100 clones and will be on display in booths 21/22.

- Aebersold, R. et al. in *Methods in Protein Sequence Analysis* (ed. Wittmann-Liebold, B.) (Springer, Berlin, 1989).
- Pappin, D.J.C., Coull, J.M. & Koester, H. in *Current Research in Protein Chemistry*, 191-202 (ed. Villafranca, J.) (Academic, San Diego, in the press).
- Coull, J.M., Pappin, D.J.C., Mark, J., Aebersold, R. & Koester, H. *Analyt. Biochem.* Submitted.
- Third Symp. Protein Soc.*, Seattle, 29 July-2 August 1989. Manual prepared for Milligen/Bioscience Protein Sequencing Forum.
- Solid-phase Methods in Protein Sequence Analysis* (ed. Laursen, R.A.) (Pierce Chem. Co., Rockford, IL, 1975).
- Wachter, E., Machleidt, W., Hofner, H. & Otto, J. *FEBS Lett.* **35**, 97-102 (1973).
- Aebersold, R., Teplow, D., Hood, L.E. & Kent, S.B.H. *J. Biol. Chem.* **261**, 4229-4238 (1986).
- Nika, H., Aebersold, R., Hood, L.E. & Kent, S.B.H. In preparation.
- Wettenhall, R.E.M., Aebersold, R., Hood, L.E. & Kent, S.B.H. *Meth. Enzym.* Submitted.
- Chang, J.Y. & Creaser, E.H. *Biochem. J.* **157**, 77-82 (1976).
- Yu, M. & Dovichi, N.J. *Analyt. Chem.* **61**, 37-40 (1989).
- Jin, S.W., Chen, G.X., Palacz, Z. & Wittmann-Liebold, B. *FEBS Lett.* **198**, 150-158 (1986).
- Cheng, Y.F. & Dovichi, N.J. *Science* **242**, 562-564 (1988).

Hybridization of Southern/Northern Blots with DNA/RNA probes



Our accurately temperature-controlled rotisserie oven gives **important advantages** as against bag: 2.4-3 ml hybridization solution. Lower background. Higher intensity of bands. Multiple hybridizations possible. Uniform distribution of solution.

**BACHOFER GmbH P.O. Box 7089
D-7410 Reutlingen W-Germany**